STUDIES OF BLOOD MONOCYTE PHAGOCYTIC FUNCTION IN RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

NIGEL P. HURST

Ph.D. Thesis

University of Edinburgh

1983

TABLE OF CONTENTS

Chapter 2 (continued)

Acknowledgments:

The author gratefully acknowledges the following for their support and assistance in performing the work undertaken in this thesis:

Professor George Nuki for his unfailing support and helpful criticism, without which this thesis would not have been possible.

The Arthritis and Rheumatism Council for financial support while the author was Research Fellow and later Clinical Lecturer at the Rheumatic Diseases Unit, Northern General Hospital.

Dr Tim Wallington of the S.W. Regional Transfusion Centre for performing tests for immune complexes including Clq binding activity and anticomplementary activity.

Dr K.C. Watson of the Dept. of Bacteriology, Western General Hospital, Edinburgh for assistance with tests for serum complement and for measurements of serum DNA binding activity.

Dr J. Webb of the Dept. of Pathology, Western General Hospital, Edinburgh for assistance with electron microscopic studies.

All the medical staff at the Rheumatic Diseases Unit, Northern General Hospital for access to patients, and all the laboratory staff for unstinting support and technical assistance.

Jean and Gail for cheerfully retrieving patients notes for me.

ABSTRACT:

This thesis examines the phagocytic function of blood monocytes in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). In vitro methods for studying the kinetics of phagocytosis by blood monocytes have been developed and validated. In preliminary studies Candida albicans opsonised with fresh serum was used as the phagocytic test particle, however since this becomes coated with a number of opsonins including IgG, IgM, IgA and complement (C3) two different particles were used in later studies to distinguish Fc and "complement" receptor mediated phagocytosis. Fc receptor mediated phagocytosis was studied using IgG coated Candida albicans while "complement" receptor mediated phagocytosis was studied using serum opsonised Saccharomyces cerevisiae. Using F(ab')2 fragments directed against Clq, C4, C3/C3c, βlH and the Fc component of IgG, uptake of the latter particle was shown to be "complement" dependent and involved mainly C3 (85%) and to a lesser extent Clq (15%). It was not established whether C3b or C3bi or both were present on the yeast and hence whether or not the CRl, CR3 or both receptors were involved.

Initial studies of monocytes from RA patients using serum opsonised Candida albicans showed a significant reduction in phagocytosis by cells from patients with cutaneous vasculitis but no change in monocytes from patients with active arthritis alone. Further studies of Fc and "complement" receptor mediated phagocytosis showed that Fc receptor mediated phagocytosis was normal but "complement" receptor phagocytosis was depressed in monocytes from patients with active cutaneous vasculitis and certain other extra-articular complications of RA. Correlations were noted between these alterations and tests for circulating immune complexes as well as reduced serum complement levels. However immunofluorescence studies did not support the possibility that these changes in phagocytic function were due to receptor blockade by immune complexes, and serum from patients with defective monocyte phagocytosis did not inhibit phagocytosis by normal monocytes. Further studies showed that changes in phagocytic function are due to the appearance of increased numbers of mononuclear phagocytes in the circulation with normal Fc but functionally absent "complement" receptors. These may represent a population of immature monocytes prematurely released from the bone marrow.

(continued overleaf)

ABSTRACT (continued)

Fc and "complement" receptor mediated phagocytosis were also studied in patients with SLE. A significant proportion of patients (9/18) had depressed rates of "complement" receptor mediated phagocytosis and only a small number (3/18) had depressed Fc receptor mediated phagocytosis. Although reduced "complement" mediated phagocytosis was correlated with increased frequency of disease manifestations there was no correlation with serum DNA or Clq binding activity, C3 or C4 levels. Serum from SLE patients with depressed monocyte phagocytosis did not inhibit phagocytosis by normal monocytes. Preliminary studies suggest that, as in RA patients, the functional changes in "complement" receptor phagocytosis may reflect an increase in numbers of mononuclear phagocytes in the blood with functionally inactive "complement" receptors. However, study of monocytes from the relatives of one of these patients showed that monocytes from the father had reduced rates of "complement" receptor mediated phagocytosis thus suggesting that there may be an inherited predisposition to this functional abnormality.

Further studies are required to elucidate which "complement" receptor is involved in these functional abnormalities in monocytes from RA and SLE patients, and whether the abnormalities reflect alterations in the number or the function of "complement" receptors. Alterations in the phagocytic function of peripheral blood monocytes may reflect receptor blockade by immune complexes or may involve alterations in the composition of the monocyte population.

GLOSSARY OF ABBREVIATIONS

ADCC	antibody dependent cellular cytotoxicity
ADP	adenosine diphosphate
ATP	adenosine triphosphate
Con A	concanavalin A
CMV	cytomegalovirus
DNA	deoxyribonucleic acid
DNP	dinitrophenol
EA	erythrocyte-antibody coated
EAC	erythrocyte-antibody, complement coated
EBV	Epstein-Barr virus
EDTA	ethylene diamine tetraacetic acid
FCS	fetal calf serum
FMLP	N-formyl-methionyl-leucyl-phenylalanine
HETE	hydroxyeicosatetraenoic acid
HLA	human leucocyte antigen
IL1	interleukin 1
LPS	lipopolysaccharide
MAF	macrophage activating factor
MIF	macrophage inhibitory factor
MHC	major histocompatibility complex
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PG	prostaglandin
PHA	phytohaemagglutinin
PMA	phorbol myristate acetate
PTFE	polytetrafluorethylene
RA	rheumatoid arthritis
RNA	ribonucleic acid
SDS	sodium dodecyl sulphate
SRS	slow reacting substance
SLE	systemic lupus erythematosus

PUBLICATIONS

The following work connected with this thesis has been or is about to be published.

Hurst N.P., Nuki G. (1981)
Evidence for defect of complement-mediated phagocytosis by monocytes from patients with rheumatoid arthritis and cutaneous vasculitis.
B.M.J.; 282; 2081-2083.

Hurst N.P., Nuki G., Wallington T. (1982)
Functional defects of monocyte C3b receptor mediated phagocytosis in rheumatoid arthritis (RA): evidence for an association with the appearance of a circulating population of non-specific esterase negative mononuclear phagocytes.
Ann. Rheum. Dis.; In press.

Hurst N.P., Nuki G., Wallington T. (1983)
Evidence for intrinsic cellular defects of Fc and "complement" receptor mediated monocyte phagocytosis in patients with systemic lupus erythematosus (SLE).
Submitted for publication.

CHAPTER 1

HISTORICAL

HISTORICAL BACKGROUND TO RHEUMATOID ARTHRITIS (RA), SYSTEMIC LUPUS ERYTHEMATOSUS (SLE), AND THE ROLE OF MACROPHAGES IN IMMUNITY

The use of the term "rheuma" is attributed to Hippocrates ("On the locations of the human body"; 4th Cent. BC) and means literally "flowing". The term was used in the humoral theory of disease, which was based on the belief that ailments were due to "defluxation of the humours", and referred to the flowing of "humours" from the brain to various parts of the body. A later, 13th century Latin derivation of the word is "gutta", meaning a "drop", which was later corrupted to "gout" and used widely as a general term to describe any form of rheumatic complaint. The specific association of "rheumatism" with joint complaints was first proposed by the French physician Guillaume Baillou (1558-1616) in a posthumously published work "The Book on Rheumatism and Back Pain". He wrote - "...one may designate the condition we are considering inexactly as rheumatism, better as a sort of precipitation like a seasickness of the vessels (which vomit), until better terms offer themselves"! Perhaps a few grains of truth can be seen hiding in his florid description.

The first clinical description of RA is usually attributed to Augustin-Jacob Landre-Beauvais (1772-1840) who in 1800 described nine women with a disease which he considered to be a variant of gout (Short 1959). He believed it was due to a "primary weakness" and that in contrast to true gout it was associated with poverty.

Several other descriptions of the clinical features of RA were made by Jean-Martin Charcot (1881), Robert Adams (1873) and Benjamin Brodie (1818) but it was Garrod (1892) who coined the term "rheumatoid arthritis" to enable a distinction to be made from true gout and other undifferentiated forms of rheumatism. Later developments include the histological description of RA nodules by Douglas Collins in 1937 (Benedek & Rodnan 1982) and the association of splenomegaly with leucopenia in RA patients described by Felty (1924).

The discovery of rheumatoid factor (RF), which forms the cornerstone of our current understanding of the pathophysiology of the disease, springs from Frank Billings hypothesis that RA is a response to chronic focal infections (Benedek & Rodnan 1982). Subsequent bacteriological research stimulated by this idea demonstrated that serum from RA patients caused agglutination of streptococci. Martin Dawson (1932) later demonstrated that this was a nonspecific phenomenon and noted that RA serum agglutinated many other organisms. A few years later Erik Waaler observed that sheep erythrocytes sensitised with anti-sera were agglutinated by 94% of RA sera (Benedek & Rodnan 1982). Waaler did not pursue his finding and Harry Rose independently made the same observation during serological studies of a Q-fever epidemic. A technician (Elizabeth Pearce) who had RA, used her own serum as a control and found that it caused agglutination of sensitised sheep erythrocytes even in high dilutions. Rose et al (1948) later developed the sheep erythrocyte reaction as a diagnostic procedure for RA.

Early descriptions of SLE focussed exclusively on the cutaneous manifestations of the disease. The first is attributed to Laurent Biett (1781-1840) who described cutaneous lupus as "erytheme centrifuge" (Jarcho 1957). Moritz Kaposi (1872) was the first to recognise the potentially life threatening and systemic nature of the disease and he described fever, adenopathy and arthritis as associated features. William Osler (1904) described the presence of renal failure in two patients with facial erythema who almost certainly had SLE, and Emanuel Libman and Benjamin Sachs (1924) later added sterile endocarditis to the list of manifestations.

The recognition of the auto-immune features of the disease stem from the demonstration of the LE-cell phenomenon in bone marrow aspirates by Malcolm Hargr aves et al (1948) an observation which gave great impetus to clinical and laboratory research. John Haserick et al (1950) showed that the phenomenon was due to a gammaglobulin while Miescher and Fauconnet (1954) found that it could be absorbed from serum by isolated nuclei and suggested that LE cell factor is an anti-nuclear antibody.

In parallel with these historical developments in the field of rheumatic disease, the scientific understanding of the nature of immunity and the bodys defence to infection had also been developing from the late 19th century onwards. Edward Jenner and Louis Pasteur had both developed empirical methods for immunisation and in 1890 von Behring and Kitasato showed that immunity to tetanus was due to a neutralising substance in the blood which

could be used to transfer immunity passively to another animal. Buchner in 1893 and Bordet in 1896 later demonstrated that there were at least two anti-microbial components in immune serum - one heat labile and the other heat stable - and it was believed that these factors entirely accounted for the anti-microbial properties of serum. In 1901 however Metchnikoff proposed that the blood phagocytic cells were the more important anti-microbial system and that serum factors merely "stimulated the phagocytes". Other studies by Almroth Wright (1903) demonstrated the complementary nature of serum and cellular factors and that the heat labile components and specific factors in immune serum were important in preparing bacteria for phagocytosis. Against this background Aschoff (1924) described the "reticulo-endothelial system" of cells which were defined functionally by their ability to take up vital dyes in vivo, and were believed to play an important role in the removal of bacteria and effete tissue. However Aschoffs "reticuloendothelial system" included a rather heterogeneous collection of cells, such as vascular endothelial cells, many of which take up material by pinocytotic nonspecific mechanisms, as well as phagocytic tissue macrophages which are capable of ingesting large particulate material. The definition of tissue macrophages and their precursors as a single system - "the mononuclear phagocyte system" - by more precise functional criteria has helped to clarify the role of these cells not only in phagocytosis of microorganisms but also their role in the clearance of immune complexes, the development of immunity and the regulation

of the immune response.

The realisation that many "auto-immune" diseases including RA and SLE are accompanied by the production of immune complexes has led to a variety of studies on the mononuclear phagocyte system and its role in the clearance of immune complexes as well as the inflammatory consequences of the interaction of immune complexes with phagocytic cells. It was with the purpose of examining the phagocytic function of blood monocytes from patients with RA and SLE that the studies described in this thesis were undertaken.

CHAPTER 2

MONONUCLEAR PHAGOCYTES

THE MONONUCLEAR PHAGOCYTE SYSTEM

The concept of the mononuclear phagocyte system (MPS) was first proposed by Langevoort et al (1970) and the cells now included in the MPS are shown in Table 1 (p12).

Characterisation of mononuclear phagocytes

A variety of morphological, cytochemical and functional characteristics may be used to define the cells of the MPS and to distinguish them from lymphocytes, reticulum cells, dendritic cells, fibroblasts, mesothelial and endothelial cells.

The characteristics used to classify mononuclear phagocytes are shown in Table 2 (p12). At the present time it is often difficult to categorise a cell with only one marker and ideally classification should be based on more than one property. The peripheral blood monocyte is a large cell about 10μm or larger in diameter with a kidney-shaped nucleus and abundant pale-staining cytoplasm. The electron microscopic appearance of the mononuclear phagocyte is characteristic - the cytoplasm contains numerous granules, prominent secretory apparatus, mitochondria and occasional phagosomes. The lysosomal granules are heterogeneous in content - some staining for peroxidase, others for enzymes such as acid phosphatase and aryl sulphatase. With maturation into the larger tissue macrophage the peroxidase content diminishes but phagocytic vacuoles become more evident (van Furth 1976). The most

used and reliable marker is non-specific esterase (Yam et al 1971) which, using α-napthyl butyrate or acetate as the substrate, gives a diffuse cytoplasmic stain which is present in most macrophages and monocytes. However the intensity of staining may vary with the species, functional and developmental state of the cell (van Furth et al 1980). Lysozyme is another good marker and can be demonstrated with immunofluorescence or immunoperoxidase labelled anti-lysozyme antibody (van Furth 1980). Cells are usually positive although sometimes staining is weak. Peroxidase activity varies with the maturity of the cell. Peroxidase positive granules are only positive in monoblasts, promonocytes, monocytes and exudate macrophages while resident macrophages are usually negative. In the latter peroxidase activity can be demonstrated in the rough endoplasmic reticulum and Golgi region by electron microscopy (Daems and van der Rhee 1980).

Functional definition of the cell by demonstrating ingestion of latex or IgG coated particles is also used but may give rise to difficulty with less mature cells which are less phagocytic and have fewer Fc and C3 receptors, (van Furth et al 1980). A variety of monoclonal antibodies to macrophage membrane antigens are currently being investigated and may prove useful in the identification of the various stages of development and differentiation in the mononuclear phagocyte.

Kinetics of mononuclear phagocytes

Mononuclear phagocytes are present in all tissues and under

normal conditions proliferate only in the marrow. The earliest member of the series to be characterised is the monoblast (Goud et al 1975) which is probably the immediate descendent of the committed stem cell. Division of this cell results in two promonocytes which are the immediate precursors of the monocyte. The human monocyte is quickly released from the marrow into the circulation where it survives for three to four days before leaving randomly to enter the tissues and become a tissue macrophage.

Various eponyms are given to tissue macrophages (Table 1) according to the site in which they are found. Isotope-labelling experiments, however, have confirmed that blood monocytes are the common source of these anatomically scattered but functionally homogenous tissue macrophages (van Furth 1980).

The kinetics of turnover of promonocytes, monocytes and tissue macrophages have been studied with isotope-labelling techniques in animals with inflammatory lesions and in normal controls (van Furth 1980, Meuret and Hoffman 1973, Whitelaw 1972, Blusse van Oud Alblas et al 1979). These studies show that while normally monocyte production in the bone marrow and tissue emigration are relatively slow, during inflammation there is a doubling in marrow monocyte output and a large efflux of these cells into the inflammatory lesion. This results from premature release of monocytes from the bone marrow and a halving of the promonocyte cell cycle time; an effect which can be blocked by inhibitors of nucleic acid synthesis such as azathioprine. It has been suggested that the tissue macrophage is capable of dividing locally at the site of

inflammation (Dannenberg 1975). However, the contribution of local cell division to the tissue infiltrate is small and is probably due to the arrival of immature monocyte precursors released prematurely from the marrow which subsequently divide once, or at most twice, at the inflammatory focus (Blusse van Oud Alblas et al 1979, van Waarde et al 1975).

Studies of human monocyte kinetics suggest that monocyte traffic is qualitatively similar in man. Using autotransfused blood cells labelled with tritiated di-isopropyl fluorophosphate Meuret and Hoffman (1973) found evidence of both a circulating and a larger marginated pool of monocytes. However, these two pools formed essentially a single kinetic unit and the ratio of the circulating and marginated pools was about 1:3.5 in both normal and diseased subjects. There is doubt as to the significance of these findings however, since the half-life of 8.4 hours for their disappearance from the circulation approximates to the half-life of 6 hours for neutrophil polymorphs and is very much shorter that the half-life of 71 hours found by Whitelaw (1972) using in vivo pulse labelling with tritiated thymidine. Whitelaw (1979) calculated a daily output of 9.4×10^8 cells/24hours and demonstrated a 13-26 hour delay before labelled cells emerged from the bone marrow, giving some indication of the cell cycle time. In monocyte distribution studies in the rat Whitelaw and Batho (1972) found that there were 25 times more mononuclear phagocytes in the tissues than in the peripheral blood and that the majority of tissue macrophages were located in the spleen and lungs. By contrast the vast majority of

neutrophil polymorphonuclear phagocytes remain in the circulation during health.

The factors regulating monocyte production in health, and the signals which stimulate the increase in monocyte production during inflammation, are not fully understood. There is good evidence for the existence of a humoral "factor inducing monocytopoiesis" (FIM), which is released both in early acute inflammation and during prolonged inflammation (van Waarde at al 1975, van Waarde 1978). This acts by reducing the promonocyte cell cycle time and, by monoblast stimulation, increases the number of promonocytes. FIM appears to be a thermolabile protein of molecular weight 18,000-24,000 which is produced by monocytes at the inflammatory site. Macrophages are also a potent source of colony stimulating factor (CSF); the generation of CSF by macrophages in an inflammatory focus may provide an important feed back amplification circuit stimulating proliferation of monocyte precursors in the marrow (Eaves and Bruce 1974). With the subsidence of inflammation a monocyte-inhibiting factor may slow monocytopoiesis by inhibiting proliferation of monoblasts (van Waarde 1978).

Two morphological varieties of the mononuclear phagocyte found in inflammatory exudate are the epithelioid cell and the multinucleate giant cell, both of which have been shown to be monocyte derived (van Furth 1980). Epithelioid cells which are found in granulomata have Fc and C3 receptors but are less phagocytic than macrophages. In the presence of strong delayed hypersensitivity reactions the epithelioid cell resembles an

activated macrophage while in the presence of low immunity it takes on a secretory constitutive appearance resembling the resident macrophage. Multinucleate giant cells are formed by fusion of exudate macrophages and two forms are recognised: the Langhans cell with a few peripheral nucleii and the foreign body type which has multiple diffusely distributed nucleii.

The ultimate fate of the macrophage is not known although there is some evidence that they migrate to lymph nodes and die there (McPherson and Steer 1980).

TABLE 1 Cells of the mononuclear phagocyte system

BONE MARROW	- stem cell; monoblast; promonocyte; monocyte
BLOOD	- monocyte
TISSUES	- histiocyte (connective tissue) Kupffer cell (liver) alveolar macrophage (lung) fixed & free macrophages (lymph node) splenic macrophages bone marrow fixed macrophage pleural & peritoneal macrophages osteoclasts (bone) microglial cell (nervous tissue) ? Langerhans cell (skin) type A synoviocyte (joint)
INFLAMED TISSUE	- exudate, exudate-resident, epithelioid, multinucleate giant cell.

TABLE 2 Identification of mononuclear phagocytes

Structure	- morphology on light, phase-contrast, and electron microscopy
Cytochemistry	- non-specific esterase, lysozyme, peroxidase
Ectoenzymes	- 5'-nucleotidase, leucine aminopeptidase, alkaline phosphodiesterase
Membrane	- Fc, C3b, C3bi receptors and macrophage antigens
Function	- immune phagocytosis, pinocytosis
Size	- cell volume

MONONUCLEAR PHAGOCYTE ACTIVATION AND MEMBRANE EVENTS

Introduction

The macrophage has a remarkable ability to respond and adapt functionally in response to inflammatory and immunological events. The basis of this physiological plasticity which is termed "activation" is not yet fully understood but a review of current understanding of this area provides a basis for discussion of macrophage phagocytic and receptor functions and their alterations in health and disease.

Activation and membrane events

The term "activation" was coined by Mackeness (1962, 1969) to describe macrophages that have enhanced ability to phagocytose and kill micro-organisms and was a characteristic particularly associated with infections such as tuberculosis and other facultative intracellular parasites. It was subsequently shown that activation can be induced either with the participation of lymphocytes or with a number of chemical agents including bacterial endotoxin, polyanions and pyran copolymer which interact with the macrophage cell membrane. Activation can thus be induced in vivo or in vitro (Table 3) (p29) (Ogmundsdottir and Weir 1980).

The term "elicited" refers to macrophages attracted to a given site by non-specific irritants and inflammatory agents such as thioglycollate and gives no specific indication of their functional

state.

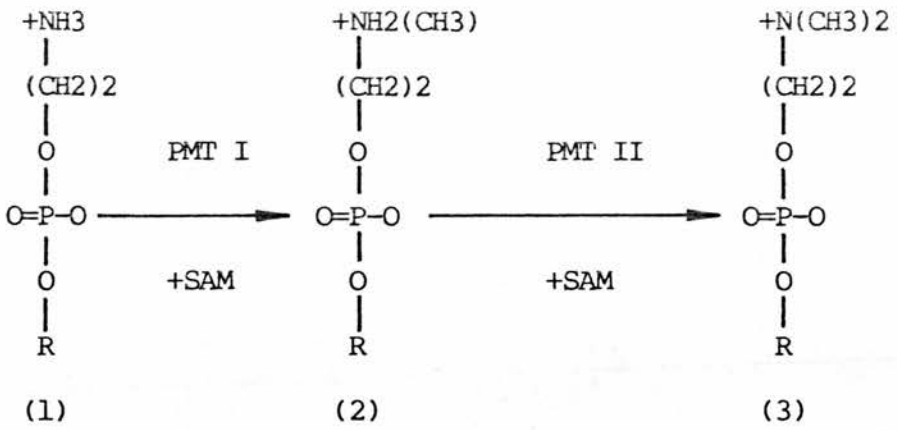
A wide variety of biochemical and biological changes occur in activated macrophages but it is likely that there is a common initiating pathway for all these events. Some examples are listed in Table 4 (p29).

Clearly for each of the known "activating" agents (Table 3) a stimulus-membrane interaction is likely to be a critical event and a consideration of the current state of knowledge of ligand-receptor interactions and their consequences is pertinent to an understanding of receptor function and "activation" in macrophages.

The lipid bilayer of cells is composed mainly of phospholipids which provide a fluid matrix for protein organisation and movement (Singer and Nicolson 1972). The lipids are asymmetrically distributed with phosphatidyl ethanolamine largely facing the cytoplasm and phosphatidyl choline facing the exterior (Rothman and Lenard 1977). Following a wide variety of receptor-ligand interactions the earliest event is rapid transmethylation of phospholipids (reviewed by Hirata and Axelrod 1980). Studies of red cell ghosts (inside out and right side out!) has revealed that following receptor stimulation a phosphomethyltransferase (PMT-1) in the cytoplasmic layer of the lipid bilayer methylates phosphatidyl ethanolamine to phosphatidyl N-monomethyl ethanolamine, and the structural change in the latter causes it to "flip" to the outer layer where it is further methylated by PMT-2 to phosphatidyl choline (the immediate precursor of arachidonic

acid). The substrate for these methylation reactions is S-adenosyl methionine (SAM) (Figure 1) (pl5).

Figure 1 Membrane phopholipid transmethylation reaction:



(1) = phosphatidyl ethanolamine

(2) = phosphatidyl monomethylethanolamine

(3) = phosphatidyl choline

SAM = S-adenosyl methionine

PMT = phosphomethyl transferase

The first methylation step causes local reduction of membrane viscosity, perhaps as a result of the lipid translocation, which has two important consequences:

a) a transient change in permeability occurs with influx of calcium (Ca++) which may trigger a number of events including synthesis of cGMP, activation of phospholipase-A2 (PLA2), and triggering of contractile elements.

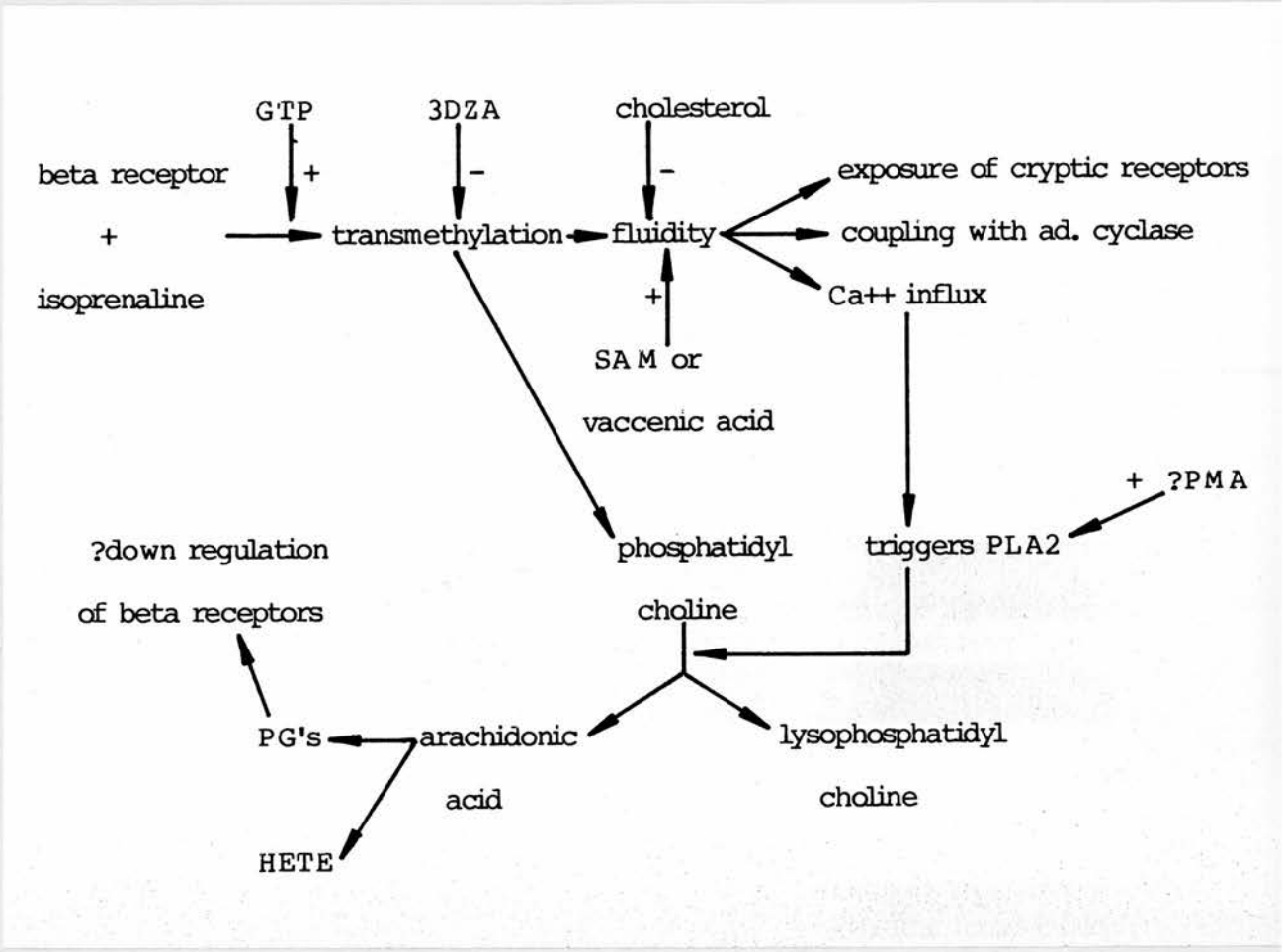
b) the increase in membrane fluidity permits increased translational movements of proteins in the lipid bilayer and therefore enhances coupling of receptors with effector enzymes such as adenyl cyclase.

These events and their metabolic sequelae have been studied in several different cell types and a brief description of these model systems will serve as a reference for subsequent consideration of receptor function and "activation" in macrophages. The key role which transmethylation plays in these systems has been demonstrated using selective inhibitors of transmethylation which include 3-deazaadenosine + L-homocysteine-thiolactone or adenosine + adenosine deaminase inhibitor + L-homocysteine-thiolactone. The effect of these compounds is to cause accumulation of adenosyl homocysteine and 3-deazaadenosyl homocysteine which compete with S-adenosyl-methionine for the transmethylation enzymes (Kredich and Martin 1977). The use of inhibitors and enhancers of adenyl cyclase, PLA2, cyclooxygenase and lipoxygenase has helped to elucidate other key steps.

Beta-adrenergic receptors

The immediate events following beta receptor stimulation with isoprenaline in model cell systems are an increase in transmethylation followed by increased membrane fluidity. The increase in fluidity results in exposure of cryptic beta receptors, permits increased beta receptor coupling with adenyl cyclase and triggers a rise in cAMP. A Ca++ influx also occurs which activates (PLA2) which in turn catalyses the degradation of phosphatidyl choline to arachidonic acid and lysophosphatidyl choline. This step is important since one of the subsequent products, perhaps PGE2, is responsible for "down regulation" of the beta receptor and provides a "braking system". Agents such as PMA which bypass the receptor and directly stimulate PLA2 cause desensitisation of the beta receptor. A variety of inhibitors and enhancing agents which operate at different levels in the pathway are illustrated in Figure 2.

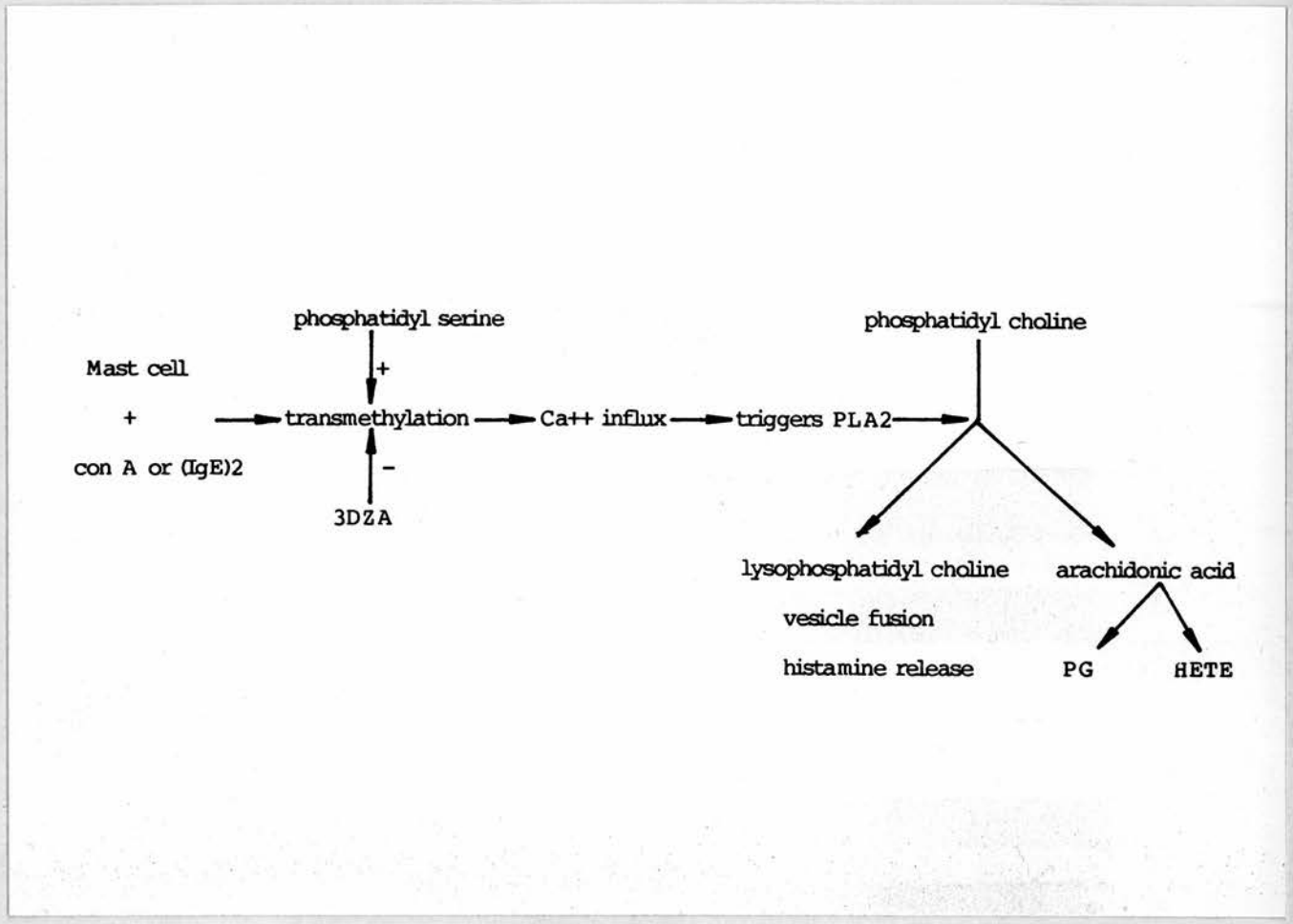
Figure 2. Beta receptors and transmethylation:



Receptors and mast cells

In mast cells, triggered by crosslinked IgE or Con A a similar sequence occurs. In this example, PLA2 activation is again important because the lysophosphatidyl choline which is generated has detergent, "fusogenic" properties which probably enhance fusion of histaminogenic granules with the cell membrane during exocytosis (Figure 3). Some typical inhibitors and enhancers of histamine release are again illustrated. Phosphatidyl serine, in which mast cells are deficient, will provide additional substrate for transmethylation and enhance histamine release.

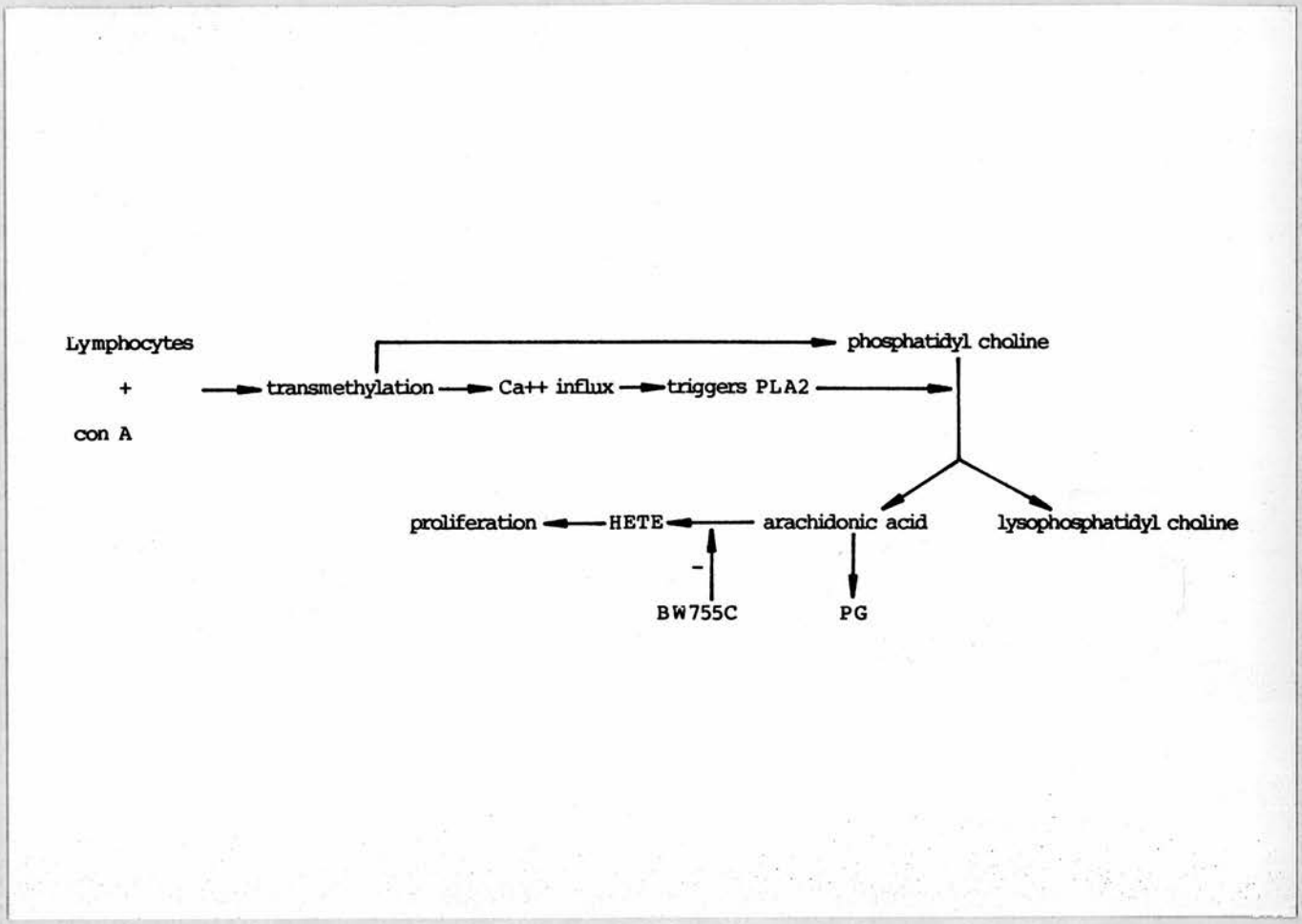
Figure 3. Dependence of IgE-mediated histamine release on transmethylation:



Con A induced proliferation of lymphocytes

Following con A induced proliferation of lymphocytes, transmethylation and influx of calcium are once again initial events. In this model the onward conversion of arachidonic acid to HETE appears to be essential for the proliferative response. Inhibition of prostaglandin synthesis via the cyclooxygenase pathway has no effect suggesting that products of the lipoxygenase pathway may be important (Figure 4). Evidence in support of this is that BW755c, which inhibits lipoxygenase, inhibits the enhancing effect of interleukin 1 (IL1 or LAF) on PHA induced thymocyte proliferation (Dinarello et al 1983).

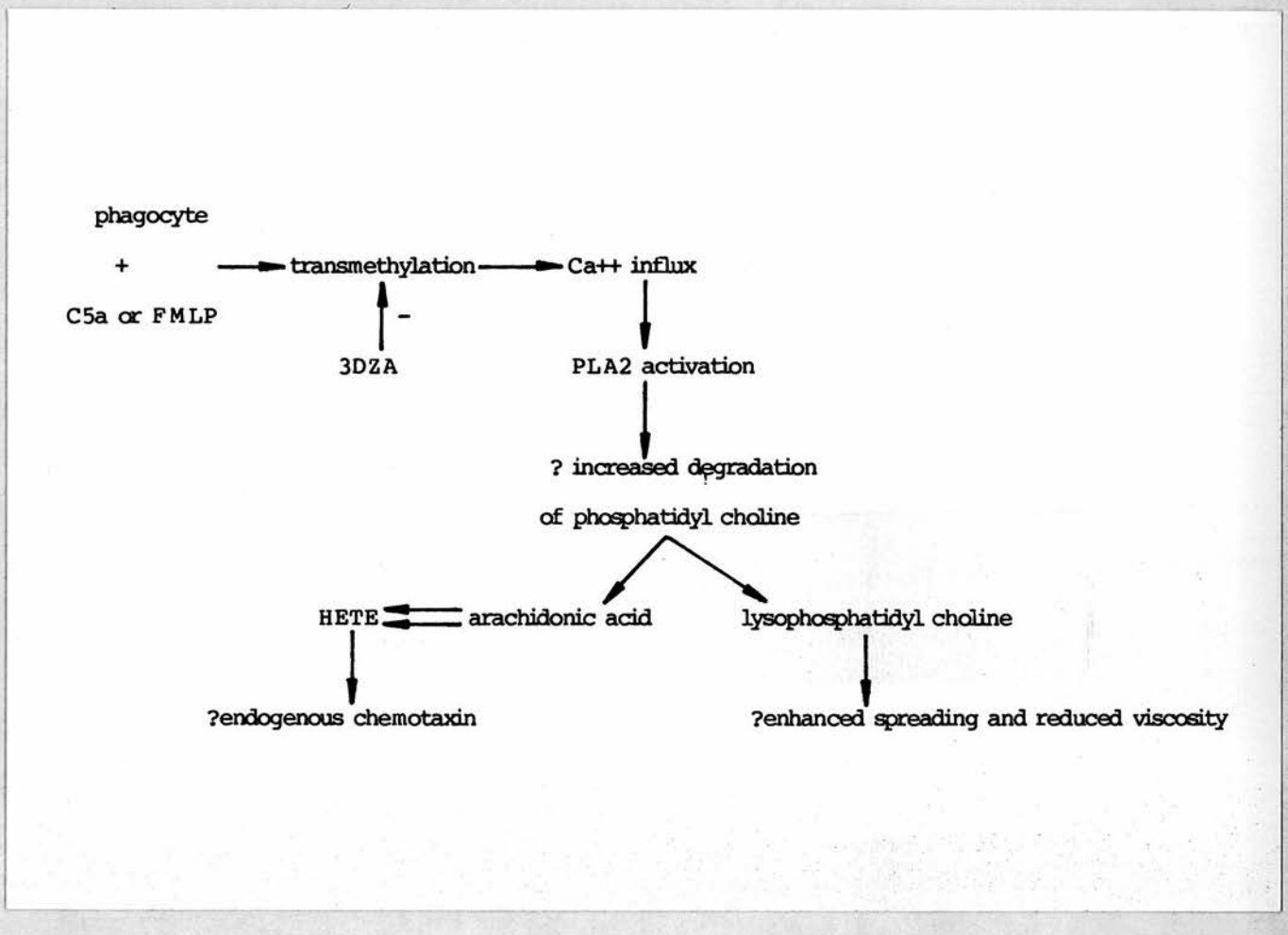
Figure 4. Lymphocyte proliferation and transmethylation:



Chemotaxis and superoxide generation

A number of workers (Pike and Snyderman 1982; Hirata et al 1979) have emphasised the importance of transmethylation in the stimulation of phagocytes by chemotactic agents such as C5a and F-met-leu-phe (FMLP). It is clear that transmethylation is a necessary step which precedes both the chemotactic response, and the associated burst of superoxide anion release and arachidonic acid (AA) production which occurs in both polymorphs and macrophages. Inhibition of transmethylation abrogates all three events. Furthermore while it appears that production of lysophosphatidyl choline and HETE play important roles in chemotaxis, the molecular mechanism which triggers superoxide release is not known. After stimulation by chemotactic agents such as C5a or FMLP there is accelerated degradation of methylated phospholipids which results in the production of large amounts of arachidonic acid (AA) and lysophosphatidyl choline. The latter with its detergent like properties may contribute to lowering the membrane viscosity, allowing it to spread. The AA is almost exclusively metabolised to HETE rather than PG which suggests an important role for endogenous chemotactic lipids in chemotaxis (Hirata and Axelrod 1980). Inhibition of PLA2 by 21-phosphohydrocortisone inhibits chemotaxis thus emphasising the importance of lysophosphatidyl choline and HETE. In addition to these events it is likely that the Ca++ influx triggers contraction of contractile elements necessary for locomotion (Figure 5).

Figure 5. Transmethylation and chemotaxis:



Another consequence of stimulation of macrophages by FMLP and C5a is enhancement of C3 receptor expression (Glass & Kay 1980). It is likely that the exposure of cryptic C3 receptors is a direct consequence of the increase in membrane fluidity which these agents induce and occurs in an analogous fashion to the mechanism described for β-adrenergic receptors (Hirata and Axelrod 1980). This hypothesis has not been formally tested.

Other recent studies (Pick and Mizel 1982) have examined the role of transmethylation in the macrophage oxidative burst in response to a number of agents including Con A, wheat germ agglutinin (WGH), FMLP, NaF, digitonin, A23187 (calcium ionophore), and phorbol myristate acetate (PMA). Their studies also demonstrate that inhibitors of transmethylation inhibit superoxide production. However it is clear that more than one mechanism may elicit a respiratory burst since different degrees of inhibition were found with different stimuli; PMA in particular was inhibited with greater difficulty than the other stimuli, suggesting that it may bypass transmethylation and activate a mechanism more closely linked to the membrane oxidase. Similarly Smolen and Weissman (1980) found that an inhibitor of PLA2, p-bromophenacylbromide, blocked the respiratory burst to some stimuli more readily than others.

Transglutaminase activity and macrophage activation

Another direct consequence of the calcium influx which follows membrane perturbation is enhanced transglutaminase activity. The

importance of this membrane enzyme is that it catalyses cross-linking and polymerisation of proteins by forming ε(γ-glutamyl)-lysine crosslinks and it has been suggested that this may play a role in the linking of receptors prior to endocytosis (Leu et al 1982). Furthermore, primary amines which are inhibitors of transglutaminase, have been shown to inhibit Fc mediated phagocytosis (Leu et al 1982) and recycling of mannose receptors during endocytosis of glycoprotein (Stahl and Schlesinger 1980). As will be discussed later, there is evidence for the existence of at least two Fc receptors with different physicochemical properties. Gergely et al (1982) have proposed that these two receptors represent polymerised and monomeric forms of the same protein and that interconversion may occur by enhancement of transglutaminase during macrophage activation.

Macrophage "activation" and membrane perturbation

Earlier studies of macrophages revealed that "activation" was accompanied by increased turnover of membrane phospholipid and calcium influx which was thought to trigger a rise in cGMP (Ogmundsdottir and Weir 1979). These observations are entirely in keeping with current views of ligand-receptor interactions. A possible mechanism for the "activation" of macrophages in the light of this model is that activating agents trigger transmethylation, enhance membrane fluidity and trigger calcium influx. These events provide a molecular basis for a number of the features of "activated" macrophages including enhanced spreading, expression of

cryptic complement receptors, coupling of complement receptors to contractile cytoplasmic elements, increased chemotaxis and secretory activity. The demonstration that MIF or LPS-activated, and oil-elicited macrophages all exhibit greatly increased transglutaminase activity is also consistent with this hypothesis (Leu et al 1982).

The role of prostaglandin secretion and rises in cAMP which often follow these events seem primarily to provide a control mechanism; if unchecked, cellular activation will proceed further to proliferation and is illustrated by systems in which inhibition of PG synthesis exposes proliferative potential (Krane 1981). The suppressive effect of prostaglandins in cytokine and lymphokine induced cellular stimulation and proliferation will be discussed later in more detail in relation to lymphocytes and connective tissue cells. Studies by Sorg (1982) on marrow macrophages in liquid culture support the view that macrophage "activation" can be linked to the cell cycle of the macrophage and that agents such as lymphokines which activate resident macrophages push them from the "constitutive" G-O phase of their cell cycle to late G1 phase where they show enhanced secretion and cellular activity such as chemotaxis and responsiveness to MIF. Agents such as PMA have similar effects stimulating the cells into late G1 phase and subsequently into S phase, causing mitosis.

The description of mononuclear phagocytes which follows will consider receptor expression and function in the light of these concepts.

TABLE 3 Agents which cause macrophage activation
(Ogmundsdottir & Weir 1980)

Chronic infectious organisms	- Mycobacterium tuberculosis Trypanosomiasis Trichinella spiralis C. parvum
Immunological agents	- lymphokines (MIF, MAF) immune lymphocytes + antigen
Others	- endotoxin, pyran copolymer BCG, group A strep cell wall zymosan

TABLE 4 Properties of activated macrophages
(Ogmundsdottir & Weir 1980; Cohn 1978)

Increased	- size, spreading, membrane ruffling
	- glucose uptake, protein synthesis
	- synthesis & secretion of neutral proteinases,
	- secretion of LAF, superoxide anion
	- content of lysosomal hydrolases
	- number of Fc receptors
	- phagocytic function of C3 receptors
	- pinocytosis, chemotaxis
	- microbial killing, cytoxicity
Unchanged	- lysozyme secretion
	- H_2O_2 generation
Reduced	- 5' nucleotidase (ectoenzyme)

MONONUCLEAR PHAGOCYTE RECEPTORS AND PHAGOCYTOSIS

Introduction

The innate capacity of the macrophage to recognise self from non-self and its ability to bind and phagocytose via lectin-like receptors and receptors for complement and immunoglobulin are illustrated by a consideration of the function of its invertebrate counterpart the amoebocyte.

The invertebrate equivalent of serum is haemolymph which contains no complement or immunoglobulin but instead contains lectins which mediate agglutination and opsonisation of foreign material. The invertebrate blood phagocyte, the amoebocyte, closely resembles the vertebrate macrophage in its morphology, its enzyme content and its ability to phagocytose foreign material.

Studies of the relationship between the invertebrate lectins and the amoebocyte show that the lectin acts not only as an opsonin but also binds to the amoebocyte membrane and provides it with specific receptors. Indeed, the slow phagocytic capacity of amoebocytes from one strain of the water snail Lymnea stagnalis can be corrected by simply incubating them for a short period in lymph from a strain with "fast eating" amoebocytes. After washing, the "slow eaters" are found to have become "fast eaters" (van der Knaap et al 1983). The presence of the lectin on the membrane of these cells has been confirmed immunocytochemically. Other studies, of starfish immunised with protein, also show a close correlation

between the appearance of specific binding lectins in the lymph and the appearance of binding sites for the protein on the phagocytic cells (Leclerc et al 1980).

In vertebrates these features have been preserved and elaborated. The macrophage still has "lectin-like" receptors in its membrane; it also synthesises the complement component C3, whose activation products C3b and C3bi show remarkably lectin-like properties and for which the macrophage has membrane binding sites. The immunoglobulins, which are of great variety, and provide a sophisticated degree of specificity, also perform cytophilic and agglutinating functions comparable to the lectins of invertebrate haemolymph, and cooperate with macrophages in a wide variety of ways to defend the integrity of the host.

In the following sections, the mechanism of endocytosis, and the structure and function of macrophage receptors for complement, carbohydrates and immunoglobulins will be reviewed.

Endocytosis

Endocytotic mechanisms can be categorised functionally on the basis of size: phagocytosis referring to the uptake of larger particles (>1micron) and pinocytosis to uptake of smaller soluble material. Perhaps a more useful categorisation is into "non-receptor mediated" endocytosis and receptor mediated or "adsorptive" endocytosis. "Non-receptor mediated" endocytosis is largely a passive process of transfer of fluid phase materials and the rate of transfer is dependent on the concentration gradient (Steinman et al 1976). Uptake occurs via so called smooth or uncoated vesicles formed by invagination of the plasma membrane. "Receptor mediated adsorptive endocytosis" includes phagocytosis of large particles as well as receptor mediated pinocytosis.

Uptake by this mechanism involves receptors located in "coated" pits, so called because of the presence of bristle like structures (Pearse 1975). On receptor-ligand binding, the pit invaginates and internalises the ligand and receptor. A wide variety of substances are taken up in this fashion and include lysosomal enzymes and glyconjugates (Stahl et al 1978) and small C3b-bearing immune complexes (Abrahamson and Fearon 1983). Larger particulate material is taken up by phagocytosis, a process which can be divided functionally into four parts:

1)The local membrane response to the particle;

2)the mechanism by which the membrane enfolds the particle;

3)the contractile mechanism;

4)the energy supply which fuels the process.

1) The local segmental response to the particle:

The segmental nature of the membrane response to a phagocytic stimulus was demonstrated by allowing erythrocytes to adhere to macrophages via a "non-phagocytic" ligand and then feeding the macrophages with a phagocytic stimulus such as latex or bacteria. The latter were promptly ingested while the erythrocytes remained attached to the exterior (Griffin and Silverstein 1974; Griffin et al 1975a). These observations suggested that the transmembrane coupling mechanisms were interacting locally with contractile mechanisms in a stoichiometric fashion rather than catalysing a diffuse membrane response.

2) The mechanism by which the membrane enfolds the particle:

The mechanism of engulfment of a particle proceeds by sequential, circumferential interaction of membrane receptors with ligands on the phagocytic particle - a process termed the "zipper mechanism" (Griffin et al 1975b; 1976). Further studies using B-lymphocytes as the test particle have confirmed that the ligands must be distributed uniformly over the surface of the particle for phagocytosis to proceed (Griffin et al 1976). Mouse B-lymphocytes were treated with anti-mouse IgG at 4°C, washed and then incubated at 25°C to allow "capping" of the IgG ligands. The capped cells were then incubated with macrophages at 4°C to allow adherence to occur. On warming to 37°C no phagocytosis occurred because the ligands were

asymmetrically distributed in the region of attachment. However on adding fresh anti-lymphocyte IgG to the macrophage-lymphocyte complexes, phagocytosis was then completed. In a second experiment, B-cells treated with anti-IgG at 4°C were washed and incubated directly with macrophages at 4°C. Since the ligand had not been allowed to cap and remained diffusely distributed on the lymphocyte surface, on warming the system to 37°C phagocytosis occurred promptly. The restriction of the ligand to the lymphocyte-macrophage attachment site when capped lymphocytes were employed, was confirmed on electron microscopy by immunoperoxidase localisation. Similar results have been obtained using other systems.

3) Contractile proteins:

The submembrane region adjacent to the particle being ingested has a thick (0.5-1.5um) meshwork of filaments from which cytoplasmic organelles are excluded (Griffin et al 1976) and contains actin (Berlin and Oliver 1978), myosin and actin binding protein (ABP) (Stendahl et al 1980).

In vitro studies have shown that actin and ABP, purified from macrophages, form gel lattices while macrophage myosin in the presence of Mg++ and ATP causes isometric contraction of the actin filaments (Stossel and Hartwig 1976). A Ca++ dependent regulator protein (gelsolin) of actin has also been identified which controls the formation of the actin-ABP lattice and probably has a physiological role (Yin and Stossel 1980). It has been proposed

that the initial membrane signal, following binding of the phagocytic particle, causes aggregation of adjacent contractile proteins (Griffin et al 1976) and results in a purely local segmental response. In support of this model, Hartwig et al (1977) demonstrated that ABP is released from the plasma membrane during phagocytosis. This, in the presence of low Ca++ concentrations (nM), causes crosslinking of actin filaments in a stoichiometric fashion and creates an ordered array of contractile proteins. Myosin in the lattice, in the presence of Mg++ and ATP, contracts the filaments, increases the shearing force and pulls in filaments from adjacent regions of lower rigidity by isotonic contraction. Since actin filaments are attached to adjacent membrane, the membrane becomes pinched upwards and outwards, forming a pseudopod which then propagates the process of attachment. A secondary response to particle attachment is a rise in cytoplasmic Ca++ which may come from the plasma membrane or the cytoplasm (Hoffstein 1979). The rise in Ca++ in the presence of gelsolin causes dissolution of the ABP-actin lattice. Thus as the region of rigid advancing membrane moves around the particle, it is followed by a secondary wave of relaxation which maintains a gradient with rigidity maximal in the tip of the advancing pseudopodium. Throughout this process, fresh insertion of membrane occurs which keeps pace with the process of internalisation (Ryter and DeChastellier 1977).

4) Energy supply for phagocytosis

Early evidence that ATP provided the fuel for phagocytosis was

based on a) the association of phagocytosis with increased oxygen consumption, glycolysis and hexose monophosphate shunt (HMP) activity and b) the inhibitory effect of fluoride and iodoacetate on phagocytosis (Karnovsky et al 1970, 1975). However it is now clear that the increased oxygen consumption, glycolysis and HMP activity are secondary consequences and that phagocytosis proceeds normally in an atmosphere of nitrogen. Furthermore, fluoride and iodoacetate are not selective and have effects on a variety of enzyme systems.

Certain observations also suggested that the simple view that ATP was the sole fuel source for phagocytosis was not correct. Stossel et al (1970) demonstrated that during phagocytosis by polymorphs there was no fall in ATP levels, and Michl et al (1976) found that reduction of ATP to less than 25% by 2-deoxyglucose did not completely prevent phagocytosis. However, since during skeletal muscle contraction there is also no fall in ATP levels, attention turned to ATP turnover during phagocytosis by measuring 32P-phosphate incorporation into ATP. These experiments surprisingly showed reduction of ATP turnover in phagocytosing cells compared with resting cells but again, by analogy with skeletal muscle, the possibility was considered that creatine phosphate was providing high energy phosphate to rephosphorylate ADP to ATP. In support of this it has been shown that macrophages have both creatine kinase (DeChatelet et al 1973) and three to five times more creatine phosphate than ATP (Silverstein and Loike 1980); during phagocytosis 90% of labelled phosphate becomes

incorporated into this large pool of creatine phosphate which therefore explains the decrease in incorporation of labelled phosphate into ATP. On the other hand since creatine is phosphorylated by ATP and is present in 3 to 5x molar excess, the specific activity of creatine on incubation of resting cells with labelled phosphate should increase more slowly than ATP. This was confirmed in experiments with resting cells which showed that the ratio of ATP:creatine-P specific activity was 10:1. During phagocytosis by resident macrophages, creatine phosphate falls by 50% but not in thioglycollate macrophages which have a much higher creatine content.

Although creatine phosphate clearly provides high energy phosphate for rephosphorylation of ATP during phagocytosis, it does not fuel ingestion in any direct manner. Thus macrophages incubated without glucose for 6 hours lose up to 90% of their creatine phosphate but retain 80% of normal ATP levels. Under these conditions phagocytosis proceeds quite normally but is accompanied by a 25% fall in ATP levels - confirming that ATP is the primary source of energy for the contractile mechanism.

Complement and complement receptors

Introduction

The complement system of proteins is functionally linked in a variety of ways with the complement receptors of phagocytes and lymphocytes. Products of the complement system activate and enhance a variety of cellular functions and conversely complement receptors have modulating effects on the complement system. As understanding of complement advances it is becoming increasingly clear that no distinct interface exists between the cellular and humoral components of complement. On the one hand complement components such as Clq (Loos 1982) and C5 (Gotze and Sundsmo 1981) are found on the membrane of macrophages where they perform important functions and conversely complement receptors modulate complement function both on cell membranes and in the fluid phase. As a preliminary to a discussion of complement receptors on macrophages, the complement system will be briefly reviewed.

The complement system

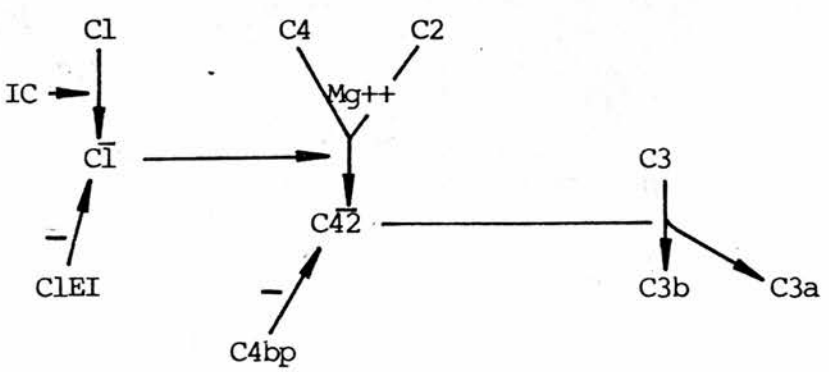
The complement system consists of a group of at least eighteen serum proteins which are involved in the host response to a variety of inflammatory and noxious stimuli. Activation of the complement pathway occurs in a manner analogous to the clotting system and results in the generation of products which are important in various inflammatory processes including phagocytosis, chemotaxis, anaphylaxis, cytotoxicity and immune regulation. Complement activation can occur via either the "classical" or "alternative" complement pathways. The classical pathway was first described in the 1960's (Lepow et al 1963) and includes the components designated C1, C2 and C4; the alternative pathway was first described in 1954 by Pillemer et al and includes properdin (P), factor B (B), and factor D (D). These two pathways converge on a final common pathway containing C3, C5, C6, C7, C8 and C9. A number of control proteins also regulate complement activation; C1-esterase inhibitor and C4 binding protein (C4bp) control the classical pathway and C3INA and β1H control the alternative pathway. There is also a poorly characterised inhibitor of the terminal sequence ("S" protein or C567INH) which may serve to inhibit formation of the terminal membrane attack sequence (MAC). The total concentration of complement lies between 2 and 3gm/l with C3 being the most abundant at about 1gm/l. The C3 molecule is a polypeptide of 180,000 daltons and consists of two peptide chains α & β (Bokisch et al 1975). Most of the early components circulate in

inactive "zymogen" form while terminal components circulate in their active form.

Activation of the classical pathway (Fig 6):

Activation of the first component of complement C1 is usually initiated by immune complexes (Porter & Reid 1979; Muller-Eberhard 1975, 1980) containing IgM or IgG. A minimum of two molecules of IgG are required to activate C1 while only one molecule of IgM is sufficient; the efficiency of IgM probably reflects the presence of its five Fc components. Not all IgG subclasses activate C1 and in humans IgG1 and IgG3 are most efficient, IgG2 is poor and G4 does not activate the classical pathway at all. C1 binds to the CH2 domain of IgG-Fc (Colomb and Porter 1975) and probably to the CH4 domain of IgM-Fc (Brown and Koshland 1977). A number of other activators of the classical pathway have been identified and include bacterial glycolipids (Morrison & Kline 1977), C-reactive protein (Claus et al 1977), and various polyanions (Raepple et al 1976).

Fig 6 The classical complement pathway:



Serum C1 consists of a Ca++ dependent complex containing one C1q,

two C1r and two C1s subunits. The C1q subunit has been shown on electron microscopy to resemble a bunch of tulips with six arms and a "collagen" like stem bound to the protein core (Loos 1982). Each arm has a binding structure which resembles a tulips head. Binding of C1q by at least two of its arms causes a conformational change which results in cleavage of C1r and C1s and generates $\overline{C1s}$ which is enzymatically active (Medicus et al 1980). $\overline{C1s}$ converts C4 to two fragments; C4a which is lost to the fluid phase and C4b which remains bound to C1 forming the $\overline{C14}$ enzyme. The C4 component of this complex binds to C2 allowing the C1 component to cleave C2 into C2a and C2b. C2b is lost to the fluid phase and may have anaphylatoxic activity (Nagasawa & Stroud 1977). C2a remains bound to $\overline{C14}$ which loses C1s to become the C3 convertase $\overline{C42}$. The liberated C1s is free to generate many more $\overline{C42}$ complexes and this therefore represents an important amplification step in the classical pathway. $\overline{C42}$ next cleaves C3 to C3b and C3a the latter having anaphylotoxic properties (Dias da Silva et al 1967). On cleavage of C3 an evanescent binding site is exposed on C3b which can bind firmly to biological surfaces via a covalent ester linkage (Tack et al 1980). The binding site probably resides in the C3d portion of the molecule which is part of the α-chain since on further cleavage of surface bound C3b (vide infra) the C3d portion is left on the surface while C3c is liberated into the fluid phase (Ruddy & Austen 1971). Binding of C3b however is inefficient and much is lost to the fluid phase. Furthermore only 10% of the bound C3b forms the C423 complex which has C5 convertase activity and

initiates the final sequence to the MAC. Within the C3 convertase ($\overline{C42}$) the C4b component serves to bind the C3 molecule while C2 performs the enzymatic function of cleaving C3 to C3b.

Control of the classical pathway

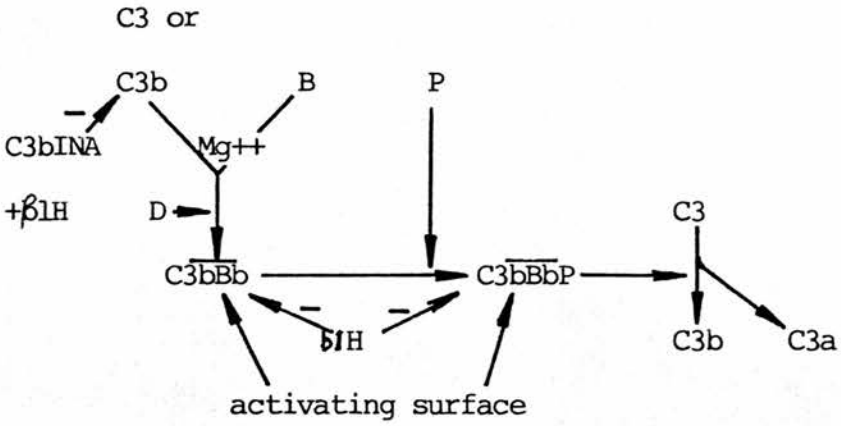
Cl esterase inhibitor (ClEI) and C4 binding protein (C4bp) are the principle inhibitors of the classical pathway. ClEI binds to Clr and Cls forming a complex and inhibits the cleavage of either C4 or C2 to form the classical C3 convertase. Genetic absence of ClEI results in episodes of uncontrolled activation, which may be triggered by infection, and are characteristically accompanied by angioneurotic oedema.

Further control of the classical pathway is exerted both by spontaneous decay of $\overline{C142}$ and its enzymatic cleavage by C4bp. The half life of $\overline{C142}$ at 30°C is only ten minutes and it decays by dissociation of C2a which leaves $\overline{C14}$ free to bind another C2. However true inactivation of $\overline{C142}$ occurs with the help of C4bp which binds C4b, displacing C2 and allowing C4b to be cleaved by C3INA (Fujita et al 1978).

Activation of the alternative pathway (Fig 7).

The alternative pathway is in a continuous state of low grade "turnover" which is closely regulated by C3bINA and βlH. Activation of this pathway reflects dysregulation of the control proteins which allows the pathway to generate more C3b which in turn forms more C3 convertase, thereby creating a self amplifying system.

Fig 7 The alternative complement pathway:



Normal low grade turnover of the pathway is due to the activities of factor D, factor B and C3b. Factor D circulates in an active state and in the presence of C3b will cleave factor B generating a $\overline{C3bBb}$ complex which has C3 convertase activity (Lesavre et al 1978). Clearly, a priori, a supply of C3b is required for this process and this is probably provided by the breakdown of C3 to C3b by slow spontaneous hydrolysis of an internal thioester bond (Vogt et al 1978). The C3 convertase ($\overline{C3bBb}$) is exactly analogous to the

classical pathway convertase. The C4b component of the classical pathway convertase binds to C3 and allows C2a to cleave it enzymatically while in the alternative pathway convertase, C3b binds C3, and Bb causes enzymatic cleavage (Fearon et al 1973). The alternative pathway convertase $\overline{C3bBb}$ which is intrinsically unstable is stabilised by binding to properdin (Fearon and Austen 1976). Properdin also protects the convertase from degradation by the action of βlH and C3bINA.

Earlier work suggested that alternative pathway activation occurred as a consequence of classical pathway activation which, by increasing the supply of C3b, increased the generation of the C3bBb convertase. However it is now clear that alternative pathway activation can occur in the absence of classical components and is triggered by a number of agents including certain xenogeneic erythrocytes, yeasts, bacteria and viruses (Fearon and Austen 1980), as well as immunoglobulins including aggregated IgE and IgA.

Many alternative pathway activators "deregulate" the control of normal low grade "turnover" of C3 by providing a protected environment for C3b and the properdin stabilised convertase $\overline{PC3bBb}$, which prevents the control proteins blH and C3INA from working. The consequence of this is rapid acceleration of $\overline{C3bBb}$ convertase generation which in turn is fuelled by the increasing amounts of surface bound C3b (Muller-Eberhard & Gotze 1972); in addition, C5 convertase activity is generated which activates the terminal sequence.

The capacity of alternative pathway activators to perform this

function is directly related to the lack of sialic acid on their surface (Czop et al 1978a). For example sheep erythrocytes which are not natural activators, become so after treatment with neuraminidase. Similarly yeast which have little or no sialic acid are excellent alternative pathway activators. Conversely, the presence of sialic acid appears to inhibit the alternative pathway by aiding the binding of βlH to C3b bound to a cell surface (Fearon 1978) of the alternate pathway

Control of the alternative pathway is exerted by inactivation of the $\overline{C3bBb}$ C3-convertase. The complex is naturally unstable and requires properdin (P) to stabilise it. βlH binds to $\overline{C3Bb}$ displacing Bb, and the α-chain of C3b in the resulting βlH-C3b complex is cleaved by C3INA, leaving the β-chain intact, to form C3bi. The resulting three peptide chains which comprise C3bi are held together by disulphide bridges and remain attached to the activating surface via the C3d part of the α-chain. Further degradation of C3bi occurs through the action of proteolytic enzymes to C3c and C3d, and C3c may be further degraded to C3e. It is likely that neutrophil elastase is one of the proteinases responsible for this activity in vivo (Carlo et al 1979). Cell membrane receptors for C3b may also play a role in the degradation of immune complex-bound C3b to C3c and C3d and this process will be discussed later.

Membrane attack complex

The binding of C3b to C5 makes the latter susceptible to

cleavage either by the classical pathway convertase C4b2a or the alternative convertase $\overline{C3bBb}$. Cleavage of C5 generates fluid phase C5a which is chemotactic and stimulates respiratory burst activity in phagocytes, and a C3bC5b complex which binds C6 forming C5bC6. The membrane attack complex then assembles onto C5bC6 with the preformed components C7, C8 and C9 without the need for further enzymatic cleavage. The final MAC is dimeric and contains six C9 molecules (Biesecker et al 1979).

Control of the final sequence

Control over the final membrane attack sequence is exerted by the action of a poorly characterised serum protein ("S" protein or C567INH) which inhibits complement dependent lysis after C567 assembly by competing with C8 for the labile binding site on C567. Its function may be to prevent reactive lysis of adjacent cells by fluid phase complement.

Biological activities

Complement has a variety of functions both in the fluid phase and when bound to surfaces. Activation of complement on membranes and formation of the MAC results in lysis of erythrocytes, tumour cells and some gram negative bacteria. The role of terminal complement components in antibacterial defence is not entirely clear and genetic absence of late components which abolishes serum bactericidal activity has been shown only to predispose to Neisseria infection (Agnello 1978). However the generation of

surface bound C3b is of much greater importance and allows recognition and binding by receptors on both phagocytes and lymphocytes. Bound C3b on bacteria is probably sufficient to trigger phagocytosis in the absence of IgG (Horwitz and Silverstein 1980) although this is not the case for C3b on IgM coated erythrocytes. There is also possibly an opsonic role for C5b which may become relatively important when levels of C3b are low (Segerling and Opferkuch 1977). Alternative pathway generation of C3b also plays a role in immune complex solubilisation and may therefore help to protect against the inflammatory effects of ICs (Miller & Nussenzweig 1975). C3b binding to lymphocyte receptors may play a role in the primary immune response to T-independent antigens by triggering the switch from IgM to IgG (Agnello 1978) while binding of immune complexes to C3b receptors on dendritic cells may be important in generating immunological memory (Klaus and Humphrey 1977). There are also receptors for C3bi, blH and C3d which will be discussed subsequently.

The three biologically active fluid phase complement molecules generated during activation are C3a, C5a and C3e. C3a and C5a are biochemically similar and it is therefore difficult to separate their biological effects. C3a is an anaphylotoxin and binds to mast cells causing degranulation with histamine, SRSA and heparin release. C5a binds to receptors on mast cells, neutrophil and eosinophil polymorphs, and macrophages stimulating chemotaxis and superoxide anion generation as well as increased membrane "stickiness" which may be involved in sequestration of neutrophils

in the lung during haemodialysis and adult pulmonary distress syndrome (Jacob et al 1980). The only function ascribed to C3e is that of mobilising marrow leucocytes and it may be important in the granulocytosis response to gram positive infections (Ghebrehiwet and Muller-Eberhard 1979).

Complement receptors

Introduction

Receptors for complement proteins are found on a wide variety of tissues (reviewed in Ross G.D. 1980) including primate erythrocytes and nonprimate platelets (Nelson 1963), neutrophil polymorphs (Anwar & Kay 1977a), macrophages (Mantovani et al 1972), monocytes (Ehlenberger and Nussenzweig 1977), B lymphocytes (Ross & Polly 1975), mast cells (Vrainen et al 1980) eosinophils (Anwar & Kay 1977a), renal glomerular cells (Schreiber & Penny 1979), synovial cells (Theophilopoulous et al 1980) and alveolar cells.

Specificity of complement receptors

Human monocytes have recently been shown, using fluorescein labelled F(ab')2 anti-C1q, to bind purified soluble C1q (Tenner and Cooper 1980, 1981). However earlier workers (Wellek et al 1976 and Gigli and Nelson 1968) were unable to demonstrate either C1 or C2 receptor activity on macrophages.

It has been known for some time that mononuclear phagocytes and lymphocytes have receptors for C3 (Ehlenberger and Nussenzweig 1977, Reynolds et al 1975, Wellek et al 1976, Huber et al 1968, Mantovani et al 1972, Lay and Nussenzweig 1968, Gigli and Nelson 1968) but more recently the specificity of these receptors has been clarified. Receptors for C3b, C3bi and C3d which are designated CR1, CR3 and CR2 respectively are now recognised on leucocytes and

their presence has been demonstrated by rosetting techniques using EAC1,4b,2a,3b (i.e. sheep erythrocytes coated with antibody, C1, C4, C2 and C3); EAC1,4b,2a,3b,β1H (i.e. EAC3b which have bound β1H); EAC1,4b,2a,3bi (i.e. EAC3bi reacted with C3bINA); and EAC1,4b,2a,3d (i.e. EAC3bi treated with protease) (Dierich et al 1982).

Using these various complement intermediates receptors for C3b, C3bi and β1H but not C3d were shown on neutrophil polymorphs, monocytes and eosinophils (Schmitt et al 1981a, 1981b). B-lymphocytes bind all these intermediates as well as C3d while erythrocytes bind only C3b (Ross et al 1973, Lambris and Ross 1981, Schmitt 1981a, 1981b). Ross and Lambris (1982) obtained similar results using either erythrocytes or fluoresceinated microspheres coated with the complement intermediates C3b, C3bi and C3d. Monocytes bound both C3b and C3bi but not C3d, and binding of C3b but not C3bi was partially inhibited by soluble C3c. Treatment of the rosetting particle with Fab anti C3c caused 100% inhibition of either C3b or C3bi binding to monocytes and neutrophils while anti C3d inhibited C3bi but not C3b binding. The binding site of C3bi to CR3 may therefore be close to but not actually on the C3c and C3d moieties while binding of C3b may be mediated through the C3c portion of the molecule. Ross and Rabellino (1979) have also shown that neutrophils and monocytes have CR3 and CR1 receptors but not the CR2 receptor for C3d, and that the binding of C3bi to phagocytes does not appear to involve the C3c or C3d regions directly. Although both anti C3d or anti C3c caused inhibition of

binding, fluid phase C3c or C3d had no effect and they therefore suggested that the binding site on C3bi may be C3e. The CR2 receptor for C3d which is found on lymphocytes on the other hand will bind not only C3d but also C3bi via its C3d portion (Ross and Lambris 1982).

It is now clear that previous work showing that phagocytic cells, including monocytes, carried receptors for C3d (Ross et al 1978, Ehlenberger and Nussenzweig 1977, Reynolds et al 1975) were in fact demonstrating C3bi receptor activity. In these studies, putative C3d intermediates were prepared by treating EACl-C3b with purified C3bINA. However cleavage of C3b by C3bINA generates C3bi (Law et al 1979, Pangburn 1977) and further cleavage into C3c and C3d requires proteolytic action for example by plasmin (Nagasawa and Stroud 1977). A further problem arose in early work on neutrophil polymorphs. It had been suggested that immature polymorphs had receptors for C3d which were lost during maturation (Ross et al 1978). As before the intermediates in these experiments were prepared using C3bINA and thus demonstrated C3bi rather than C3d receptors. However the interpretation was further complicated by the fact that mature polymorphs secrete elastase which cleaves C3bi to C3d (Taylor et al 1977); the apparent loss of receptor activity was due to cleavage of C3bi to C3d on the rosetting particle. When protease inhibitors are included in the test system, C3bi receptors (CR3) can be demonstrated on both mature and immature polymorphs (Ross and Lambris 1982).

A further problem may arise when attempting to demonstrate C3b

(CR1) receptors on leucocytes as a result of endogenous secretion of βlH and C3bINA. Both monocytes (Whaley 1980) and B lymphoycytes (Ross 1980) secrete βlH and C3bINA which will cleave C3b on indicator cells to C3bi and thus interfere with the rosetting assay. This can be prevented by inhibition of synthesis of C3bINA and βlH with EDTA and sodium azide or by inhibiting these proteins with antisera (Ross 1980). Another approach is to use an alternate pathway activator such as yeast or zymosan. Zymosan generates large amounts of C3b on its' surface by providing a protected "microenvironment" for both the C3b molecule and the properdin stabilised convertase (P,C3b,Bb) (Fearon and Austen 1977). C3b is activated by deregulation of the normal low grade turnover of the alternate pathway and this is achieved by inhibiting the inactivation of C3b by C3bINA and βlH and by inhibiting the βlH catalysed dissociation of the stabilised C3 convertase (P,C3b,Bb).

Using rosetting techniques, receptors for βlH have been demonstrated on lymphocytes, neutrophils, eosinophils and monocytes (Schmitt et al 1981a, 1981b, Dierich and Schmitt 1980). Dierich & Schmitt (1980) have shown that binding of EAC3b to Raji cells is considerably enhanced by βlH and that some putative C3b receptor binding can be attributed to contamination by βlH. Engagement of B lymphocytes by aggregated or complexed blH causes release of C3bINA from B cells which may result in cleavage of bound C3b to C3bi as described above (Ross 1980).

Receptors for C5b have been demonstrated on human neutrophils which may play a role in phagocytosis (Segerling and Opferkuch

1977) but no comparable receptor has been demonstrated on macrophages.

Molecular characteristics of complement receptors

Perhaps the earliest isolation of an erythrocyte membrane component which bound to complement was that performed by Hoffman (1968). He isolated a substance from erythrocyte membrane on agarose gel which inhibited complement dependent lysis of erythrocytes. Subsequently, Fearon (1979) isolated a glycoprotein of 205,000 daltons (gp205) from human erythrocyte membranes using a non-ionic detergent Nonidet-P40 (NP40) which was found to inhibit alternate pathway convertase activity by disassembling the C3b,Bb complex and augmenting the cleavage of C3b to C3bi by C3bINA. Antibody to gp205 was found to inhibit C3b dependent rosette formation on human B lymphocytes, granulocytes and monocytes suggesting that gp205 might be the C3b receptor. Anti-gp205 also precipitates a glycoprotein molecule from solubilised membrane obtained from B cells, neutrophils and monocytes (Fearon 1980) which again has a molecular weight of 205,000 on SDS polyacrylamide gel electrophoresis. Scatchard analysis of Fab2 anti-gp205 binding to various cell types gave values of 950 molecules/erythrocyte; 21,000/B-cell; 57,000/neutrophil; and 48,000/monocyte. These numbers are comparable to the number of C3 receptors detected using C3b dimers generated by trypsin cleavage of C3 (Arnaout 1981).

Similar results on human erythrocyte CR1 receptors have been obtained by others using the same procedures (Dobson et al 1981b, Dierich et al 1981). Thus, isolated radioiodine labelled CR1 bound either C3b or C4b, and on SDS PAGE the receptor protein had a molecular weight of 195,000 daltons. Furthermore antibody to CR1

blocked CR1 rosetting by lymphocytes, monocytes and neutrophils but not CR2 or CR3 rosetting .

Using KBr solubilisation of human erythrocyte membranes a smaller glycoprotein of 55,000 daltons (gp55) has been obtained which also binds C3b and inhibits C3b dependent rosetting (Mussel et al 1982). The gp55 moiety has a major carbohydrate portion which appears to play an important role in the binding of C3b; thus while treatment with periodic acid causes loss of C3b binding potential, treatment with trypsin does not (Dierich et al 1982). Furthermore C3b binding to CR1 can be inhibited by several monomeric sugars including D-glucose, D-galactose, D and L-fucose and D-xylose but not L-glucose, L-galactose, L-xylose or D-mannose suggesting that C3b has lectin like properties in binding to its receptor. The antigenic relationship between gp55 and gp205 suggests that gp55 may in fact represent the binding site of gp205 (Dierich et al 1982). Although the isolated glycoprotein does not depend on its protein content for C3b binding, the intact receptor in the cell membrane is trypsin sensitive (Lay and Nussenzweig 1968; Doughaday and Douglas 1976) and its integrity in situ must therefore depend on its polypeptide component.

Schneider et al (1981a) have purified a complement receptor from rabbit alveolar macrophages. Radio-iodinated rabbit alveolar macrophage membrane was solubilised with NP40 and the glycoprotein purified by adsorption to Sepharose-C3 or C3b. A single peak was obtained with a molecular weight of 64,000 daltons which had greater affinity for C3b than for C3.

A C3d binding glycoprotein has also been isolated from human Raji cells and identified as the CR2 receptor (Lambris et al 1981). The glycoprotein was isolated initially from Raji cell culture media and shown subsequently to be of membrane origin. SDS PAGE and gas-liquid chromatography indicate that it has a molecular weight of 72,000 daltons and contains a single polypeptide chain. The molecule binds sheep RBC-C3d but not C3b and antibody to the glycoprotein inhibits rosette formation with EC3d but not EC3b. CR2 was found only on B-cells and not on T-cells, monocytes, neutrophils or erythrocytes.

Genetic polymorphism of the complement (CR1) receptor

Recent studies (Dykman et al 1983) of erythrocyte CR1 isolated from single individuals have demonstrated that the protein shows structural polymorphism. CR1 was purified by C3-sepharose affinity chromatography from 125 radio-iodine labelled erythrocytes solubilised in NP40. Subsequent autoradiographic analysis of the purified protein on SDS PAGE revealed three distinct patterns. In type a individuals there was a single band of 190,000 daltons, in type b a single band of 220,000 while in type c both 190,000 and 220,000 bands were found. Both bands had an associated minor band which was 15,000 daltons greater than the main band. Study of 33 individuals gave a frequency of 70% type a, 3% type b, and 27% type c. Family studies demonstrated a pattern of inheritance consistent with the presence of two codominant alleles.

Ultrastructure of complement receptors

Early studies suggested that complement receptors were randomly distributed over the surface of endotoxin induced macrophages (Kaplan 1977). More recent studies showed that C3b receptors on human neutrophil polymorphs and monocytes are distributed in clusters (Petty et al 1981) and unlike Fc receptors show no lateral mobility within the plane of the cell membrane (Petty et al 1981, Michl et al 1979). Others using indirect immunofluorescence and antibody to CR1 receptors on neutrophil polymorphs confirmed that CR1 are non-randomly distributed in clusters on the cell membrane at 0°C, but at 37°C the antibody-ligand rapidly disappears suggesting that endocytosis has occurred (Fearon et al 1981). These observations prompted more detailed ultrastructural studies (Abrahamson and Fearon 1983) of the distribution of CR1 on monocytes and polymorphs using ferritin labelled Fab2 anti-C3b receptor antibody. These studies show that CR1 receptors are located in clusters on the cell membrane surface and after ligand binding they are rapidly internalised by adsorptive endocytosis via so called "coated pits" or cytoplasmic invaginations. Ligand internalised in this fashion was delivered finally to lysosomes and no uptake was seen via large phagosomal structures.

An intact cytoskeleton is also of importance in the function and expression of C3 receptors. Inhibitors of microtubules such as colchicine and vinblastine as well as microfilament inhibitors such

as the cytochalasins inhibit rosette formation with complement sensitised particles (Atkinson et al 1977b). The rosetting of complement receptors is also temperature dependent, being maximal at 37°C and almost undetectable at 4°C (Lay and Nussenzweig 1968). However as mentioned above, complement receptors are detectable on the membrane at 0°C using immunofluorescence and inhibition of rosetting probably reflects reduction in membrane fluidity rather than reduced receptor expression. Cytoskeletal inhibitors may also prove to inhibit rosetting by interfering with membrane contact rather than by preventing receptor expression.

Function of complement receptors

The functional importance of complement receptors parallels that of the complement system in which a number of inherited defects have been described and which predispose to serious infection and immune complex diseases. For example deficiencies of classical pathway components C1r, C1s, C4 and C2 are all associated with the development of systemic lupus erythematosus; C3 deficiency (Alper et al 1976; Alper 1970) is associated with life threatening recurrent infection, C6 and C8 deficiency with gonococcaemia, and C3bINA deficiency with severe recurrent infection (Table 10,p145). Quite apart from the opsonising and chemotactic activities of these proteins it has also been shown that extracellular IgG and complement are necessary for killing of opsonised microorganisms which have already been ingested by monocytes and presumably act by stimulating surface complement and Fc receptors (Leijh et al 1979). B-lymphocyte C3b receptors may also play a role in the induction of antibody responses to certain antigens (Pepys 1976).

Endocytosis and binding via complement receptors

Although it is generally agreed that C3b promotes adherence of sensitised particles such as erythrocytes or bacteria to phagocytic cells, there has been controversy over the role of complement in mediating endocytosis. It is now clear that the function of C3 receptors has to be related not only to the maturation and functional state of the phagocyte but also to the chemical nature

of the particle and the presence or absence of other ligands.

Lay and Nussenzweig (1968) demonstrated adherence of C3b coated sheep erythrocytes to mouse peritoneal macrophages and showed that the receptor is trypsin sensitive. Griffin et al (1975a) also using resident mouse peritoneal macrophages and sheep erythrocytes demonstrated binding but not ingestion. Ehlenberger and Nussenzweig (1977) also reported binding but not ingestion of C3 coated erythrocytes to human monocytes but did demonstrate that C3b had a synergistic effect on Fc mediated ingestion. Similarly van Furth et al (1979) demonstrated that while nearly 100% of human monocytes bound, only 16% ingested C3 sensitised sheep red cells. Newman et al (1980) have found that while freshly separated human monocytes do not ingest C3 coated sheep red cells, after seven days in culture they acquire the ability to ingest via C3b or C3bi receptors. Using different test particles ingestion via C3 receptors can be demonstrated without manipulation of the phagocyte. For example E. coli opsonised with complement is phagocytosed by human monocytes (Horwitz 1980) and the uptake of heat aggregated IgM by mouse and guinea pig macrophages occurs in the presence of complement (van Snick and Masson 1978); Kijlstra et al 1978). Schreiber et al (1982) likewise have shown that E.coli coated with C3b or C3bi are bound and ingested by human neutrophil polymorphs while similarly coated sheep erythrocytes are not. Furthermore, opsonisation of bacteria with agammaglobulinaemic sera results in both phagocytosis and killing (Williams and Quie 1971), although this may of course be attributable to serum components

other than complement.

Although there has been dispute over the ability of C3b receptors alone to mediate phagocytosis of large particles there is now good evidence that C3b receptors mediate adsorptive endocytosis (Fearon et al 1981 ; Abrahamson et al 1983) and thus may provide a mechanism not only for clearance of complement coated immune complexes but also of fluid phase C3b generated at sites of inflammation thus assisting in the limitation of inflammatory damage. Similar clearance mechanisms may exist for Clq, C3bi, C3d and C5a (Jesaitis et al 1983).

The discrepancies between these different systems reflect differences in the maturity and state of activation of the phagocyte as well as the nature of the test particle since bacteria and yeast but not sheep red cells may provide additional phagocytic ligands (vide infra) (Czop et al 1978a & 1978b).

Synergism between complement and Fc receptors

A number of workers have reported that complement receptors enhance Fc receptor mediated phagocytosis and have shown that in the presence of complement much smaller amounts of IgG are needed to mediate ingestion (Ehlenberger and Nussenzweig 1977). Shaw and Griffin (1981) also provide evidence that while C3 augments Fc mediated ingestion of sensitised red cells it does not participate in the ingestion process. In these experiments erythrocytes coated with IgG and complement were allowed to adhere to mouse peritoneal macrophages at 4°C. The remaining unengaged Fc receptors were then

blocked with an anti-macrophage serum which did not interfere with C3 receptor function (Bianco et al 1975). After warming to 37°C phagocytosis did not occur. Others (Michl et al 1979) have studied the modulation and functional interrelation of Fc and C3 receptors using a different system and obtained contrary results. Normal or inflammatory mouse peritoneal macrophages were allowed to adhere to glass cover slips coated with either DNP-antiDNP IgG complexes or DNP-antiDNP IgG-C3b complexes and then the effect on binding of IgG or C3b sensitised erythrocytes to the macrophages observed. After adhering to IgG complexes a reduction in Fc binding and ingestion was seen but surprisingly adherence to the IgG-C3b coated surface reduced only C3b receptor binding and had no effect on Fc binding of IgG coated erythrocytes. Thus C3b appeared to mask the Fc portion of the DNP-IgG complex making it ineffective as a ligand for receptor binding. This contradicts the hypothesis of Shaw and Griffin (1981) and suggests that in the presence of complement, binding and ingestion occurs via C3b not Fc receptors. A further unexpected finding was that layering of macrophages onto DNP-IgG-C3b had no effect on the ability of resident macrophages to bind complement coated red cells but profoundly inhibited binding and ingestion by inflammatory macrophages. This observation may reflect differences in C3b receptor-cytoskeletal linkages in resident and inflammatory macrophages.

Synergism between complement receptors and "non specific" ligands

In 1968 Lee noted that desialation of sheep and ox

erythrocytes with neuraminidase increased their adhesion to mouse macrophages and promoted their ingestion in the presence of nonimmune mouse serum i.e. desialation had two effects, it allowed recognition of the erythrocyte by a macrophage membrane receptor and promoted opsonisation by nonimmune serum presumably via complement activation. The significance of these findings has only recently been appreciated (Czop et al 1978a, 1978b) and indirectly has led to the identification of the leucocyte and erythrocyte C3b receptor. Czop et al (1978) have shown that particles which are natural activators of the alternate pathway of complement are ingested by human monocytes in the absence of complement or immunoglobulin whereas "non-activators" are not ingested. Thus zymosan, rabbit erythrocytes, mouse C57B and CBA erythrocytes which are all alternate pathway activators are all ingested. Furthermore, the rank order of ingestion of these erythrocytes exactly parallels their rank order of lysis by complement (Rabbit > mouseC57B > mouse CBA); sheep and guinea pig erythrocytes which are not activators of the alternate pathway are neither lysed by complement nor ingested by monocytes. Ingestion of particles by this mechanism can be inhibited by trypsin using amounts of trypsin which are insufficient to alter Fc receptor mediated uptake or C3b receptor binding. Further work has shown that the ability of these particles to activate the alternate pathway and to be ingested is inversely related to the amount of sialic acid on their surface. Thus desialation of sheep erythrocytes by neuraminidase or chemical conversion to heptulosonic acid increases their "ingestability" in

a dose dependent fashion. Further, while opsonisation of untreated sheep erythrocytes with C3b has little or no effect on ingestion, after desialation, C3b has a synergistic effect on phagocytosis of these particles. The mechanism of deposition of C3b on the surface of alternate pathway activators has also been related to sialic acid content. Using preformed $\overline{C3bBb}$ convertase a direct relationship was found between deletion of sheep erythrocyte sialic acid residues, inhibition of the βlH and C3bINA mediated C3b degradation and decreasing ability of βlH to cause decay of the $\overline{C3bBb}$ convertase. Thus for alternate pathway activators a direct link is established between the absence of sialic acid, amplification of C3b generation by deregulation of βlH and C3bINA and recognition and ingestion by monocytes. These observations suggest that the plasma proteins of the alternate pathway which are responding to the regulatory effects of alternate pathway activators are prototypes of the monocyte membrane receptor proteins involved in the ingestion of these activators (Czop et al 1978a). Further relationships between C3, macrophages and alternate pathway activators are discussed in the next section.

Role of complement receptors in cellular secretion

C3b receptor stimulation causes lysosomal enzyme release from macrophages and it has been suggested that this may play an important role in a number of chronic inflammatory conditions. In vitro it has been shown that many stimulants of chronic inflammation such as immune complexes, dental plaque, zymosan and mouldy hay dust have two properties in common - they activate the alternate pathway of complement with cleavage of C3 to C3b, and they stimulate prolonged lysosomal enzyme release from macrophages (Cardella et al 1974; Schorlemmer et al 1977). These effects appear to operate in parallel, are dose dependent and are not associated with macrophage cytoplasmic enzyme release or death. Furthermore C3b itself may also stimulate lysosomal enzyme release thereby amplifying the original signal (Schorlemmer and Allison 1976; Schorlemmer et al 1976). In addition the mononuclear phagocyte itself synthesises a number of complement components, including factor B, factor D, Properdin (P), C2, C3, C4, C5, C3bINA and βlH (De Ceular et al 1980; Whaley 1980; Ruddy and Colten 1974; Littman and Ruddy 1977) and some of the enzymes which the macrophage secretes are able to cleave complement components. For example, plasminogen activator, produced in large quantities by activated macrophages (Gordon et al 1974; Unkeless et al 1974; Gordon et al 1978) converts plasminogen to plasmin. The latter can cleave C3 to C3b, and C5 to the chemotaxin C5a, the net result being self sustaining generation of inflammatory stimuli. A further

interrelationship between alternative pathway activators, macrophage secretion and endogenous C3 synthesis has been suggested recently (Schorlemmer et al 1981). In these studies F(ab')2-anti-C3 added to serum free cultures of macrophages and alternative pathway activators was shown to inhibit secretion of hydrolases in a dose dependent fashion. This strongly suggests that conversion of endogenous C3 to C3b causes stimulation of hydrolase release via C3b receptors. Similar inhibition was obtained with added exogenous bIH and C3bINA. It is also possible that a similar mechanism is involved in the spontaneous phagocytosis of unopsonised alternative pathway activators or alternatively that activation of endogenous C3 on the macrophage membrane triggers ingestion without a requirement for C3b receptors. These possibilities have not been explored, but are a further potentially interesting interaction of macrophages and complement.

Oligomers of C3b also modulate the release of other enzymes and have been shown to inhibit the zymosan induced release of histaminase from neutrophils (Melamed et al 1981 (51)).

Complement receptors also play an important role in stimulating the oxidative burst of phagocytic cells and evidence for the involvement of Clq, C3b, C3bi and C5a receptors has been found. Particle bound Clq but not monomeric fluid phase Clq has been shown to stimulate chemiluminescence responses in neutrophil polymorphs but the response is weak compared to C3 and Fc receptor stimuli (Tenner and Cooper 1982, Schreiber et al 1982). Chemiluminescence responses are also obtained with sheep

erythrocytes coated with C4b (Schreiber et al 1982).

However interest in the ability of complement to activate the respiratory burst has centered mainly on the question of whether C3b, C3bi and β1H receptors are capable of stimulating oxidative metabolic responses without a requirement for other ligand binding. Newman and Johnston (1979) used trypsin generated C3b bound to sheep erythrocytes (EC3b) to examine whether C3b alone would stimulate a respiratory burst and degranulation in human neutrophil polymorphs. EC3b alone did not stimulate release of superoxide anion, glucuronidase or lysozyme. If small amounts of IgG were also present on the red cell, neutrophil stimulation occurred and it was clear that C3b had a synergistic effect on Fc receptor mediated activity. They confirmed the specificity of C3b receptor binding of EC3b both by blocking with fluid phase C3b and by the reduction in rosetting after treatment with C3bINA. However Goldstein et al (1976) using nonphagocytosable Sepharose 4B beads coated with C3b found that CR1 stimulation alone released superoxide from neutrophil polymorphs but degranulation and lysosomal enzyme release only occurred if IgG were present. The effect on superoxide release was amplified if IgG was present. In these experiments Sepharose was opsonised with normal serum which results in opsonisation with C3b but also in the adsorption of small amounts of IgG; the IgG was removed by boiling in 2M Nacl and the absence of IgG confirmed by immunofluorescence. This data therefore has been questioned on the grounds that it would be difficult to exclude the presence of small amounts of IgG on the C3b coated

beads. However it was also shown that superoxide release was completely blocked by pretreating the C3b beads with F(ab')2 anti-C3. Schopf et al (1982) have also demonstrated activation of human monocytes both by oligomeric C3b and by purified βlH. C3b was prepared by trypsin cleavage of C3 and any contaminating βlH removed by affinity chromatography. Both βlH and C3b stimulated chemiluminescence in monocytes but neither caused an increase in NBT reduction and in fact C3b appeared to have an inhibitory effect on the latter. They suggested that C3b may act by stimulating release of βlH from monocytes, an effect which has been previously observed in lymphocytes (Dierich et al 1982). Schreiber et al (1982) using purified C3b, C3bi and C3d have shown that sheep erythrocytes coated with C3b or C3bi elicit chemiluminescence from neutrophil polymorphs in a dose dependent fashion but C3d does not. The specificity of binding was confirmed by inhibition with purified monomers and, in experiments using C3bi, protease inhibitors were included to inhibit neutrophil elastase degradation of C3bi to C3d. C3b opsonised zymosan also elicited chemiluminescence in the absence of IgG and they demonstrated that C3b polymers but not monomers caused chemiluminescence. The finding that monomeric C3b has no effect is in line with the finding of Fearon et al (1981) that for C3b mediated adsorptive endocytosis monovalent interactions are insufficient. Dobson et al (1981a) have also compared the ability of complement components bound either to sheep erythrocytes (EC3b, EC3bi) or to zymosan (ZymC3b, ZymC3bi) to release superoxide from human neutrophils. Neither EC3b nor EC3bi

caused superoxide release while ZymC3b and ZymC3bi did. The ZymC3b stimulated release was completely inhibited by Fab anti-C3c or anti-blH. However Fab antiCR1 only slightly inhibited superoxide released by ZymC3b or ZymC3bi whereas crosslinking of CR1 receptors by Fab2 anti-CR1 did not elicit any superoxide release i.e. the stimulation of CR1 alone is insufficient. They conclude therefore that C3b/C3bi mediated release of superoxide by ZymC3b/ZymC3bi involves a structure on zymosan which is absent on sheep erythrocytes.

The bulk of the evidence at present seems to indicate that engagement of CR1 and CR3 receptors by polymeric C3b or C3bi may elicit a chemiluminescence response but is insufficient without additional ligands, such as the Fc portion of IgG or sugar residues on yeast, to elicit superoxide release. The ease of demonstration of chemiluminescent responses in part reflects the sensitivity of chemiluminescence techniques and may not always represent a significant biological event.

Functions of the isolated complement receptor glycoprotein

A search for a red cell membrane protein which would inhibit and thus prevent damage to erythrocytes by complement (Hoffman 1968, Fearon 1979) subsequently led to the identification of the human CR1 receptor (Fearon 1980). This glycoprotein (gp205) has been shown to inhibit alternate pathway activation by disassembling the $\overline{C3bBb}$ convertase and augmenting the cleavage of C3b by C3bINA. Other studies have shown that gp205 is also a cofactor for inactivation of C4b by C3b/C4bINA (Iida & Nussenzweig 1981). A glycoprotein gp55 which may represent the head portion of gp205 also has regulatory effects and enhances the binding of βlH to C3b (Dierich et al 1982). Since receptor glycoprotein is shed from cells into the fluid phase (Dierich 1976, Lambris et aal 1981) these molecules may have important regulatory effects in the extracellular environment as well as on membrane surfaces.

Moreover, recent studies by Medof et al (1982) have shown that soluble or membrane bound CR1 catalyses C3bINA dependent degradation of immune complex bound C3b to C3c and C3d and results in release of C3b-immune complexes from the CR1 receptor. The reaction occurs independantly of blH and represents a novel function for the immune adherence receptor.

Modulation of complement receptors

The expression of C3b receptors by monocytes can be enhanced by a number of agents including lymphokines, and chemotaxins (FMLP, C5a, endotoxin, HETE); is influenced by physical factors such as temperature; and alters with the maturity of the cell. In view of the observations of Hirato and Axelrod (1980), Ogmundsdottir and Weir (1979) & (1980), and Sorg (1982) discussed previously it is highly likely that these diverse factors exert their effects by: (1)activating membrane transmethylation reactions, thereby increasing membrane fluidity, exposing cryptic receptors and enhancing receptor coupling with contractile elements (2)triggering calcium influx and calcium dependent cytoplasmic biochemical events.
Additionally these agents also trigger PLA2 activation which, by generating AA, lysophosphatidyl choline and products of the lipoxygenase and cyclooxygenase pathway exert further enhancing and inhibiting effects. In most instances the effects on complement receptor expression are reversible and occur maximally within 30 minutes emphasising that new protein synthesis is not required. Some examples of complement receptor modulation will be discussed.

Whereas few murine promonocytes (30%) express C3b receptors up to 90% of blood monocytes do so (van Furth et al 1980). In addition, the phagocytic capacity of the C3b complement receptor increases with maturity in culture and in the presence of inflammatory stimuli (i.e. thioglycollate elicited vs resident macrophages)(Bianco et al 1975; Newman et al 1980). The poor

expression of complement receptors on promonocytes may reflect the low synthetic and activation state of the cell in the early stages of the monocyte cell cycle as has been observed for other biochemical activities (Sorg 1982). The enhancement occurring with maturation in vivo and in culture presumably in part reflects receptor synthesis but also reflects alterations in membrane fluidity which facilitate linkage of complement receptors to cytoskeletal elements. This is supported by the observations of Michl et al (1979) who showed that while complement receptors were not mobile in the plane of the membrane of resident macrophages, they became mobile in thioglycollate or "inflammatory" macrophages.

The rapid changes in complement receptor expression occurring in response to a number of other stimuli are unlikely to be attributable to new receptor synthesis. A number of workers have shown that chemotactic agents cause considerable enhancement of complement expression in monocytes, neutrophils and eosinophils over a period of 30 minutes. Anwar & Kay (1977b & 1978) showed that eosinophil chemotactic factors (ECF) including the tetrapeptides val-gly-ser-glu and ala-gly-ser-glu, histamine and its major metabolite imidazole acetic acid enhanced C3b and C4b receptors on human eosinophils. Similarly the N-formylated peptides F-met-leu-phe, F-met-met-phe and F-met-phe also enhanced C3b receptor expression on human neutrophils and monocytes whereas the unformylated peptides had neither chemotactic nor receptor enhancing effects (Kay et al 1979). Glass & Kay (1980) extended these findings and showed that other chemoattractants including

casein, supernatants from C parvum and from PHA stimulated lymphocytes enhanced monocyte complement receptor rosette formation. These agents have little or no effect on Fc receptor expression. The effect of all these agents was dose dependent, time courses showed maximal effects within 30 minutes and after removal of the stimulus, it was reversible. Lipid chemotactic products of the lipoxygenase pathway of arachidonic acid metabolism have similar effects. Goetzl et al (1980) showed that hydroxyeicosatetraenoic acids (HETE) increased eosinophil and neutrophil complement receptors but had no effect on Fc receptors. Conversely the generation of HETE via the lipoxygenase pathway during stimulation by other chemotaxins such as C5a may therefore enhance the cells responsiveness and provide an amplifying mechanism.

The role of lymphokines in this process has also been studied. Although lymphokines generated by primed T-cells which were incubated with macrophages and antigen had no effect on resident macrophage complement receptors (Griffin & Griffin 1979), macrophages which had ingested IgG containing complexes stimulated release of a different lymphokine from T-cells. This lymphokine had no effect on macrophage Fc receptor expression but did stimulate complement receptor mediated ingestion in resident macrophages (Griffin and Griffin 1980).

Fearon and Collins (1983) have used immunofluorescence and dimeric radioiodine labelled C3b as probes of C3b receptors on neutrophils and monocytes to examine the effect of separation

procedures and temperature on C3b receptor expression. They found that neutrophils separated at 4°C had far fewer C3 receptors (5,500/cell) than those separated at 20°C (21,000/cell). Cells incubated for a further 30 minutes at 37°C had 38,000 receptors/cell. Furthermore, neutrophils kept unseparated in whole citrated blood at 37°C without centrifugation showed no increases in C3 receptors. They also confirmed that both C5a and F-met-leu-phe caused 8 to 10 fold increases in receptor expression.

The biological importance of the effect of chemotactic agents and lymphokines is that they provide an important mechanism for enhancing the bacterial recognition and phagocytic mechanisms of monocytes and neutrophils moving down a chemotactic gradient towards an inflammatory focus. From a practical viewpoint the observations of Fearon and Collins (1983) highlight some of the artefacts that may arise during clinical studies of receptor function.

"Lectin like" receptors

Mononuclear phagocytes have "lectin-like" receptors for carbohydrate residues which provide a simple but specific mechanism for the recognition of altered "self" molecules as well as foreign "nonself" molecules. These receptors have been implicated in the recognition, binding and clearance of serum glycoproteins (Stahl et al 1978, 1980; Ashwell & Morell 1974; Stockert et al 1976; Lunney & Ashwell 1976; Baynes & Wold 1976; Winkelhake & Nicolson 1976; Achord et al 1977; Shepherd et al 1981; Townsend & Stahl 1981; Schlesinger et al 1980.), lysosomal enzymes (Stahl et al 1978, 1980; Schlesinger et al 1976, 1978; Achord et al 1977), desialated erythrocytes (Czop et al 1978a & 1978b; Kolb et al 1978; Nagamura & Kolb 1980; Jancik 1978; Schlepper-Schaffer et al 1980), IgM-antigen immune complexes (Day et al 1980), tumour cells (Weir et al 1979; Schlepper-Schaffer et al 1981) and bacteria and yeast (Czop et al 1978b; Sung et al 1983; Ogmundsdottir & Weir 1976 & 1978; Freimer et al 1978; Glass et al 1981; Weir et al 1981). Evidence is also accumulating for the role of specific carbohydrate recognition mechanisms in the immune response and in particular in macrophage T-cell interactions (Muchmore & Blaese 1979; Stewart et al 1982; McKenzie et al 1977) and cell mediated cytotoxic reactions (Muchmore & Blaese 1979).

Specificity of receptors for glycoprotein and lysosomal enzymes

The outer sequence of carbohydrate chains of most mammalian glycoproteins consists of a triad of sugars with a terminal nonreducing sialic acid residue linked to D-galactose which in turn is linked to N-acetyl-D-glucosamine. Asialoglycoproteins, which have a terminal galactose are virtually absent from mammalian serum and if infused are very rapidly cleared from the circulation. Ashwell and Morell (1974) originally proposed that a specific recognition mechanism existed for the terminal galactose to account for the very rapid clearance of these glycoproteins in vivo and it is now known that this receptor is present on the hepatocyte. The receptor will recognise and bind asialated, galactose bearing erythrocytes and tumour cells as well as glycoprotein and also has some specificity for glucose conjugated glycoprotein (Schlesinger et al 1980). From a phylogenetic viewpoint it is interesting to note that avian and reptilian species have high circulating levels of asialoglycoproteins and lack the galactose specific receptor. However, avian and probably reptilian species have a hepatic receptor for N-acetyl-D-glucosamine which is the residue exposed after removal of galactose (Lunney & Ashwell 1976).

Subsequently, recognition mechanisms for lysosomal enzymes and glycoproteins have also been identified in the macrophage which are predominantly D-mannose and L-fucose specific but which also bind D-glucose and N-acetyl-D-glucosamine terminated glycoprotein. It might therefore be considered that the macrophage receptor represents a phylogenetically older system than the mammalian

hepatic galactose receptor. A number of workers have shown specific receptor mediated clearance in vivo of mannose and N-acetyl-D-glucosamine bearing glycoproteins (Stockert et al 1976, Baynes & Wold 1976, Shepherd et al 1981, Schlesinger et al 1980). Others have shown that lysosomal glycosidases such as β-glucuronidase, β-galactosidase, α-mannosidase and N-acetyl-D-glucosaminidase are cleared by the same receptor (Stahl et al 1980, Schlesinger et al 1976, 1978, Achord et al 1977). For example, Sclesinger et al (1978), comparing the clearance of radioiodine labelled lysosomal enzymes and glycoproteins, found that β-glucuronidase, ribonuclease B and agalacto-orosomucoid, all of which have either a terminal mannose, N-acetyl-D-glucosamine or both, are cleared by Kuppfer cells whereas asialo-orosomucoid with a terminal galactose was cleared mainly by hepatocytes. It has also been shown that L-fucose, D-mannose and N-acetyl-D-glucosamine terminated glycoproteins compete for the same macrophage receptor (Achord et al 1977, Shepherd et al 1981) and that the receptor will recognise glucose conjugated to neoglycoprotein (Stahl et al 1978, Shepherd et al 1981). The order of specificity found by Shepherd et al (1981) was :

L-fucose & D-mannose > N-acetyl-D-glucosamine & D-glucose > D-xylose.

The receptor system has also been implicated in the clearance of IgM-antigen immune complexes from the circulation. Unlike most serum glycoproteins with long half-lives, IgM has mannose residues and ought in principle to be rapidly cleared by hepatocyte and

Kupffer cell receptors. It has therefore been suggested that steric inaccessibility of the sugar residues is responsible for the long half life of the immunoglobulins (Day et al 1980). Experiments with IgM and IgM-BSA complexes have provided evidence for this hypothesis and show that when IgM binds to antigen the mannose residues become exposed and mediate clearance of the complex (Day et al 1980). Thus the rapid clearance of IgM-BSA complexes from the circulation was inhibited by mannan and ovalbumin but not by asialofetuin, dextran or depletion of serum complement. Furthermore while IgM does not normally bind Concanavalin A, it does do so after complexing with antigen, and both Con A binding and RES clearance can be abolished by prior treatment of the complex with α-mannosidase. Thus binding of antigen appears to induce a conformational change in IgM resulting in exposure of mannose oligosaccharide residues which mediate receptor clearance from the circulation.

Isolation and preliminary characterisations of the mannose/N-acetyl-D-glucosamine/fucose binding protein by affinity chromatography have now been carried out. A receptor isolated from rat liver consisted of a single band with a molecular weight of approximately 30,000 on SDS PAGE (Townsend and Stahl 1981). Binding of radioiodine labelled mannan to the putative receptor protein was specific and saturable and could be inhibited by mannose, N-acetyl-D-glucosamine and L-fucose but not by galactose or mannose-6-phosphate. The binding of neoglycoprotein conjugates was also studied; those with a terminal mannose, N-acetyl-D-glucosamine

or L-fucose were bound 10,000 times more strongly on a molar basis than the free monosaccharide; glycoprotein with terminal galactose was not bound. The relative strength of binding observed with neoglycoprotein conjugates points to highly stereospecific binding requirements. A similar protein of molecular weight 31,000 has been isolated from rabbit liver and showed a similar spectrum of inhibition with saccharides (Kawasaki et al 1978).

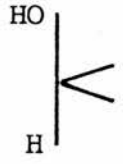
Specificity of receptors for desialated erythrocytes

As described above, there is considerable evidence for uptake of galactose terminated glycoproteins via a hepatocyte receptor and uptake of mannose/fucose/N-acetyl-D-glucosamine terminated lysosomal enzymes and IgM-antigen complexes via a macrophage receptor. However, there is also evidence for distinct, predominantly galactose specific receptors on macrophages which bind desialated erythrocytes in vitro and are not inhibited by mannose or N-acetyl-D-glucosamine.

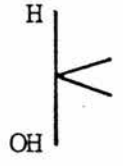
Although in general the presence of sugars enhance cell-cell interactions by attractive van der Waals forces the presence of terminal sialic acid on cell membrane oligosaccharides confers a strong hydrophilic, negative electrostatic charge which is involved in cell-cell and cell-protein repulsion (Nir & Anderson 1977). Erythrocytes desialated with neuraminidase agglutinate rapidly in vitro at room temperature and, in vivo, become coated with complement and immunoglobulin and cleared from the circulation (Jancik 1978). Similarly, studies of the metastasising potential of tumour cells has demonstrated that the presence of sialic acid on the tumour cell potentiates metastasis whereas cells with N-acetyl-glucosamine or D-galactose have low metastatic potential (Fogel et al 1983). The ability of macrophages to recognise desialated erythrocytes via the exposed galactose residue has been investigated in some detail (Czop et al 1978a, 1978b, Kolb et al 1978, Nagamura & Kolb 1980, Schlepper-Schaffer et al 1980).

Czop et al (1978a), investigating this phenomenon, found that

the degree of sialation of erythrocytes and other alternate pathway activators was inversely related to their ability to be spontaneously phagocytosed by monocytes and to activate the alternative pathway of complement. They also found that the macrophage recognition mechanism for the desialated erythrocytes was trypsin sensitive, suggesting that a membrane protein was responsible for facilitating the interaction with terminal D-galactose. Subsequent work confirmed that neuraminidase treated rat erythrocytes are bound by a receptor on rat Kuppfer cells which can be inhibited by D-galactose and N-acetyl-D-galactosamine but not by a number of other sugars. These workers (Nagamura & Kolb 1980, Schlepper-Schaffer et al 1980) have since examined in more detail the stereochemical requirements for binding by this receptor using a variety of monosaccharides and their substituted derivatives; oligosaccharides; and glycoproteins with defined terminal sugars. In general the sugars which inhibited binding of desialated erythrocytes shared the galactose configuration at carbon-4. i.e.:



whereas noninhibiting sugars had the opposite stereochemistry:



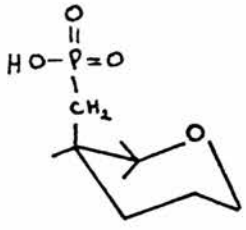
These sugars are summarised in Table 5.

TABLE 5 Inhibitors of lectin-like receptor

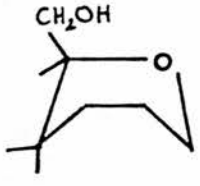
Inhibitors	Noninhibitors
D-galactose	D-mannose
D & L-fucose	D-glucose
glucose-6-phosphate	D-fructose
N-ac-D-galactosamine	N-ac-D-glucosamine
lactose	D-galactosamine
lactulose	
melibiose	
D-galactosyl-albumin	
D-glucosyl-albumin	
asialo-orosomucoid	

Exceptions to this generalisation were glucose-6-phosphate and glucosylated-albumin which, although apparently possessing the wrong stereochemistry, did inhibit binding. The authors do not offer an explanation for these exceptions. However the ability of glucose-6-phosphate to block the receptor must be attributable to the phosphate substitution. The only difference between D-glucose and D-galactose is at the carbon-4 position where in the energetically favoured chair conformation of galactose the C4-OH is axial while in glucose it is equatorial:

Esterification of glucose C6-OH by phosphate could provide the missing axial -OH which is presumably vital to receptor binding:



A similar effect might be postulated in glucose conjugated albumin where, if the glucose were held in the less favourable chair conformation by the albumin molecule, the C4-OH would be oriented in the axial position and might then be available for binding to the receptor site.



Conversely, D-galactosamine did not exhibit the expected inhibition of receptor binding whereas the N-acetyl derivative did. Again stereochemical considerations suggest that the presence of a negatively charged -NH2 group might inhibit approximation of the C4-OH to its binding site.

In addition there is evidence for a second galactose specific receptor on "activated" macrophages which does not bind glucose-albumin conjugates (Nagamura and Kolb 1981).

Specificity of receptors for microorganisms and tumour cells

There is also evidence that so called nonspecific binding and ingestion of a variety of gram positive and gram negative organisms is mediated in part by "lectin-like" receptors in the phagocyte membrane which recognise "foreign" sugars on the bacterium (Ogmundsdottir & Weir 1976, 1978; Freimer et al 1978). These receptors have been demonstrated on phagocytes from different species and of different types including macrophages, neutrophil polymorphs, eosinophils and monocytes. However study of this phenomenon is complicated by a number of other factors. Some organisms themselves have "lectin-like" receptors for binding to tissue cells. E. coli for example binds to mannose residues on epithelial cells (Ofek et al 1977) and a "lectin-like" molecule has been isolated from C. parvum which also has specificity for mannose (Bqgg et al 1981). Others such as Cryptococcus neoformans (Kozel & Mastrioanni 1976) and encapsulated Pseudmonas species (Oliver & Weir 1983) release cell wall material which can block the phagocytic recognition system. Furthermore the bacterium may have more than one sugar exposed on its surface and therefore engage multiple phagocyte receptors.

Ogmunsdottir & Weir (1976) demonstrated that binding of C. parvum to peritoneal exudate cells could be inhibited by a number of monosaccharides including glucose, glucosamine, fructose, N-acetyl-D-glucosamine, galactose, fucose, glucuronic acid and rhamnose. All of these except fucose and rhamnose were detected in the bacterial cell wall but the latter may have been competing with

sterically similar sugars. It should be noted that glucose and galactose did not cause synergistic inhibition which suggests that they are competing for the same binding site. Further studies (Freimer et al 1978) with a variety of gram positive and negative organisms demonstrated binding which could be inhibited by glucose, galactose or both providing the sugars were present on the bacterial cell wall. Using mutants of S. typhimurium and K. aerogenes which have various saccharide deletions from their surface, a clear relation was established between the ability of monosaccharides to block binding and their presence on the bacterial cell wall. The only exception was mannose which did not inhibit even though it is present on each of the Salmonella strains tested. This may be due to the additional side linkage of mannose to abequose which could interfere with its steric accessability to receptor linkages.

The importance of mannose binding however has been demonstrated in the phagocytosis of yeast. Lung macrophages bind and ingest T. krusei and zymosan via a receptor which is mannose/glucosamine specific (Warr 1980), and Sung et al (1983) have demonstrated that ingestion of zymosan by peritoneal macrophages is inhibited by mannans which are a major constituent of yeast cell wall (Northcote and Horne 1952). They also showed that blockade of the mannose receptor had no effect on Fc or C3 receptor function.

Evidence has also been found for binding of macrophages to tumour cells (Weir et al 1979) particularly during the growth phase

before they become confluent. As in the case of bacteria, galactose, glucose, galactosamine and glucosamine were found to be the principal inhibitors of binding of macrophages to target cells. Likewise Schlepper-Schaffer et al (1981) have shown galactose specific receptors on Kuppfer cells and hepatocytes which will bind tumour cells.

The pattern of inhibition obtained with monosaccharides differs in some respects from that obtained with the less complex glycoproteins and desialated erythrocytes and suggests that lectin-like binding sites in addition to those described for galactose and mannose/fucose/N-ac-D-glucosamine may be involved. It should also be noted that inhibition by monosaccharides never exceeded about 30% emphasising the importance of other physico-chemical interactions between bacteria and macrophages (Capo et al 1979).

Muchmore and Blaese (1979) have made related observations on the cytotoxic effects of monocytes on xenogeneic target erythrocytes. Testing the hypothesis that macrophages are interacting with target cell carbohydrates they found that for a given target, a predictable spectrum of oligosaccharides would inhibit cytotoxicity, and that a different spectrum was found for different target species. Thus the monocyte displayed the ability to recognise a range of sugars and the pattern of inhibition was dictated by the saccharide components of the target cells. Others however have found evidence against this mechanism of target recognition (Vose et al 1983).

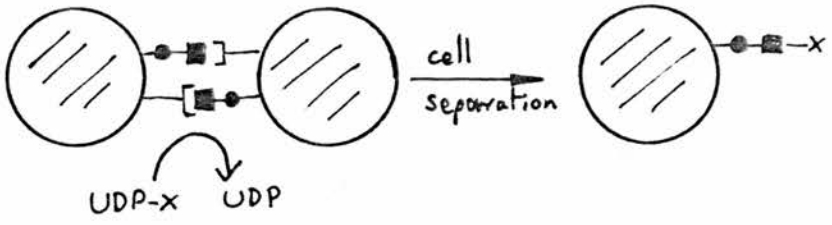
Relation between "lectin-like" receptors and Ia (DR) antigens

Several lines of evidence point to an important role for oligosaccharides in the functioning of Ia molecules both in the initial contact of macrophage with antigen and in subsequent macrophage /T-cell interactions and induction of immunity.

Firstly there is evidence that there are both carbohydrate and protein antigenic components to the MHC antigens (McKenzie et al 1977, Higgins et al 1980, Parish et al 1976a, 1976b, 1978, Mann & Muchmore 1980); secondly, proliferative responses of immune T-cells to antigen bearing macrophages may be inhibitable by simple carbohydrates (Muchmore & Blaese 1979); thirdly, macrophage binding of unopsonised bacteria may be mediated in part through Ia antigens with lectin-like properties (Stewart et al 1982); fourthly, human Ia (DR) molecules may bind to carbohydates (Mann & Muchmore 1980).

Previously it has been suggested that the regulation of cell-cell interactions in a wide range of contexts, including growth and differentiation of tissues, proliferative responses of normal and tumour cells, and in pathophysiological events such as adhesion of platelets to damaged tissue, are mediated by cell membrane bound glycosyl transferases (Shur and Roth 1975).

The general model they have proposed for binding and separation of cells involves recognition of "acceptor" oligosaccharide molecules by specific glycosyl transferases;



In this model, initial binding of "receptor" transferase to an "acceptor" molecule is followed by monosaccharide transfer from the appropriate nucleotide and is followed in turn by cell separation. Since transferases are highly specific for both sugar donor and acceptor a unique saccharide chain is constructed and specified by the available transferases. This model allows cells to mutually create as well as to recognise self.

The application of this model to the function of the MHC locus was suggested by the observation that MHC controlled antigens have both protein and carbohydrate constituents. McKenzie et al (1977) demonstrated that certain defined Ia molecules (Ial, 3, 7 & 15) had a major carbohydrate determinant and that recognition of these molecules by anti-Ia antibody could be blocked in a highly stereospecific fashion by certain monosaccharides. The "immunodominant" monosaccharides for these Ia antigens were Ial/N-acetylglucosamine; Ia3/a-D-galactose; Ia7/L-fucose; Ial5/N-ac-D-glucosamine. These observations have been extended and confirmed and have led to the hypothesis that the MHC gene products

are glycosyl transferases which construct the MHC carbohydrate antigens (Parish et al 1976a, 1978, Higgins et al 1980). The possibility that the protein MHC antigens are these transferases has not yet been tested. This model of immune macrophage/T-cell and T-cell/T-cell interaction proposes the glycosyl transferase as the anti-self receptor and has a second "antibody" like receptor for recognition of foreign antigen.

Further evidence to support this view is suggested by the observations of Muchmore and Blaese (1979) who found that immune T-cell proliferative responses to antigen presenting macrophages could be inhibited by some carbohydrates but not by others. The phenomenon was not attributable to nonspecific effects and a different set of sugars were required to block PHA induced proliferation. Studies of DRw alloantisera reactivity with human monocyte DR antigens have demonstrated that certain sugars inhibit the cytotoxicity of antisera to some DR antigens and not others and conversely anti-DR sera blocked the attachment of these sugars to the monocyte. The authors conclude that the DR antigen appears to have receptor like properties for certain saccharides.

Indirect clinical evidence for the ability of saccharides to interfere with the regulation of cell mediated immunity (CMI) is illustrated by chronic mucocutaneous candidiasis (Fisher et al 1978). These patients often have high antibody levels to candida but depressed CMI, and the cellular defect does not appear to be primary since it improves after anti-fungal therapy. Moreover it has been shown that serum from some patients with active infection

contains polysaccharide antigens which block specific candida-antigen induced lymphocyte proliferation.

Stewart et al (1982) have also provided evidence that Ia molecules may play a role in the unimmunised state by contributing to binding of unopsonised bacteria. It was shown that binding of S. albus to peritoneal macrophages could be blocked by anti-Ia region allo-antibodies and that glucose or galactose which also inhibit binding did not cause synergistic inhibition with anti-Ia. Thus it appears that glucose and galactose are competing with anti-Ia for the Ia molecule binding site.

Evidence for a role for saccharides in lymphokine-monocyte interactions has also been suggested. Studies of MIF showed that α-L-fucose blocks recognition by MIF of a saccharide determinant on monocytes and that treatment of monocytes with fucosidase abolishes the response to MIF (Remold 1973). Further studies have shown that both L-rhamnose and L-fucose inhibit MIF as well as the action of chemotactic lymphokines. They had no effect on the action of C5a or C3a (Amsden et al 1978).

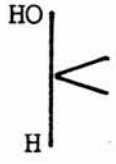
Molecular characteristics of "lectin-like" receptors

A number of workers have studied the chemical characteristics of macrophage lectin receptors and preliminary isolations have been achieved.

Binding to the receptor is reduced by trypsin and pronase (Stahl et al 1978, 1980, Ogmundsdottir & Weir 1978, Weir et al 1979) as well as by mild oxidation with periodate suggesting that it is a glycoprotein. Moreover, the inhibitory effect of periodate which removes two terminal carbon atoms from sialic acid and oxidises the C7 hydroxyl to aldehyde, can be reversed with borohydride (BH_4^-) which will reduce the C7 aldehyde back to an alcohol group; this suggests that -OH groups play a role in binding. Other sugars such as galactose might be expected to provide OH groups and this is supported by the inhibitory effect of β-galactosidase. Neuraminidase treatment however enhanced binding to lectin-like receptors but this may be due to reduction of the net negative electrostatic charge conferred on the membrane by sialic acid.

Calcium ions were also shown to be important for binding to the receptor and this supports the view that lectin receptor binding involves interactions between -OH groups via Ca^{++} bridges (Stahl et al 1978, 1980, Shepherd et al 1981, Nagamura & Kolb 1980, Ogmundsdottir & Weir 1976, Weir et al 1979, Warr 1980). The importance of Ca^{++} bridging between carbohydrates in cell surface events such as adhesion and agglutination has been studied widely (Cook & Bugg 1975). Furthermore the formation of Ca-OH bridges

between carbohydrates in an aqueous environment has highly stereospecific requirements which would therefore confer specificity on receptor binding. The stereospecificity of lectin receptor binding with respect to D-galactose has already been discussed (Nagamura & Kolb 1980, Schlepper-Schaffer et al 1980). However the stereospecificity of binding of sugars to Ia molecules has also been studied (McKenzie et al 1977). Ia3 was found to bind D-galactose as well as a variety of structurally related sugars which enabled the stereochemistry of the interaction to be examined. The binding of D-galactose and related oligosaccharides and glycosides to Ia3 was shown to be heavily dependent on the galactose C4 conformation i.e.



and to a lesser extent on the alcohol group at C5. The orientation of the C2 -OH group was also shown to play a minor role.

Preliminary isolation and characterisation of the mannose/fucose binding protein has been achieved. Townsend and Stahl (1981) isolated a 30,000 molecular weight protein band on SDS PAGE of rat liver extract and demonstrated typical receptor binding, saturation and inhibition characteristics. Kawasaki et al (1978) have also isolated a similar 31,000 molecular weight protein on PAGE from rabbit liver.

Fc receptors

Introduction

The enhancement of macrophage phagocytosis by serum opsonins had been recognised for many years (Briscoe 1908) before the discovery that this was related to the presence of antibodies (Vaughan 1965) and, in particular to macrophage receptor sites for the Fc component of IgG (Howard and Benacerraf 1961; Berken and Benaccerraf 1966). IgG-Fc receptor binding has been identified in a wide variety of cells including human monocytes (Huber and Fudenberg 1968), lymphocytes (Nussenzweig 1974, Moeller 1981), neutrophil polymorphs (Messner and Jelinek 1970), eosinophils (Anwar and Kay 1977), platelets (Pfueller et al 1977a), basophils (Segal et al 1981), as well as non-haemopoietic tissues such as placenta (Johnson et al 1981) and intestinal epithelium. Binding of cytophilic IgE to mononuclear phagocytes has also been described (Joseph et al 1978).

In mononuclear phagocytes the Fc receptor facilitates phagocytosis of foreign (Reynolds 1974) and autologous material (Kay 1975), mediates ADCC (Haskill and Fett 1976 , Shaw et al 1978), and stimulates secretory activity such as superoxide anion generation (Goldstein et al 1975), release of prostaglandins (Kurland and Bockman 1978; Bonney et al 1980), acid hydrolases (Cardella et al 1974) and neutral proteinases (Unanue 1976, Passwell et al 1980). The biological activities and character of

cytophilic antibodies has recently been comprehensively reviewed (Leslie and Alexander 1979).

Specificity of Fc receptors

The presence of Fc receptors on cells has been demonstrated mainly by erythrocyte rosetting assays using antibody sensitised erythrocytes (EIgG). Ultrastructural studies have also been carried out using peroxidase or ferritin labelled immune complexes (McKeever et al 1976; Papadimitriou 1972); others have used fluorescence conjugated labels (Schwartz et al 1976). Numbers and affinity of Fc receptors can be demonstrated using binding and competitive inhibition of radiolabelled immunoglobulins or antigen-antibody complexes (Arend and Mannik 1973, Unkeless and Eisen 1975).

A number of studies have suggested that macrophages have two IgG-Fc receptors distinguished by different physicochemical properties, different sensitivities to proteases and lipases and different specificities for subclasses of cytophilic antibody and antibody-antigen immune complexes. In general immune complexes bind with higher affinity than monomeric IgG (Segal and Horwitz 1977) but as discussed below, monomeric IgG can none the less interfere with binding of immune complexes. Probably the earliest observation to suggest the presence of two different Fc receptors was that of Kossard and Nelson (1968). They noted that erythrocytes sensitised with "early" antisera following immunisation formed rosettes with macrophages via a trypsin sensitive receptor whereas if they were sensitised with "late" hyperimmune sera the binding could no longer be prevented by treating the macrophages with trypsin. Askenase (1974) confirmed this observation and found that only the trypsin

sensitive binding could be blocked by IgG2a subclass antibody.

Subsequent evidence which supports the view that there are least two Fc receptors is based on the demonstration of selective cytophilic binding of certain IgG subclasses, competitive inhibition studies using myeloma proteins of defined subclass, the demonstration of mutant cell lines with selective loss of certain receptor characteristics, and partial purifications of the putative receptor proteins.

Affinity studies have shown that human monocytes bind subclasses IgG1 and IgG3 with equal and high affinity (Huber & Fudenberg 1968, Huber et al 1971, Alexander et al 1978a, Abramson et al 1970, Hay et al 1972) and IgG4 with somewhat lower affinity. The association constant for IgG1 (10^{8} M^{-1}) is high and all available receptor sites will be occupied by monomeric IgG under physiological conditions (Alexander et al 1978a). Thus rosetting of EIgG by human monocytes is easily inhibited by low concentrations of monomeric IgG (Alexander et al 1978a). Mouse macrophages bind monomeric IgG2a and IgG2b but not IgG1, IgG3, IgA or IgM (Unkeless 1977, Lane et al 1980, Anderson & Grey 1977, 1978, Unkeless & Eisen 1975, Segal & Titus 1978, Unkeless 1979, Unkeless et al 1978, Walker 1976) and bind complexed or aggregated IgG1, IgG2a or IgG2b strongly and with equal affinity (Heusser et al 1977). Guinea pig macrophages bind monomeric and complexed IgG2 (Alexander et al 1978a, Leslie & Cohen 1974, 1976) and to a lesser extent IgG1 (Leslie et al 1976).

A number of studies have shown that binding of immune

complexes to mouse macrophages (Steinman & Cohn 1972), guinea pig macrophages (Howard & Benacerraf 1961), rabbit macrophages (Arend & Mannik 1973) and human monocytes (Lobuglio et al 1967) is not inhibited by prior trypsinisation of the macrophage. Unkeless (1977) using a kinetic system to study binding of myeloma proteins to mouse macrophages (Unkeless and Eisen 1975) confirmed that while binding of rabbit IgG-antigen complexes to mouse macrophages is trypsin insensitive, cytophilic binding of monomeric IgG2a is trypsin sensitive thus suggesting that two types of receptor are present. Further studies (Unkeless et al 1978) used mutant macrophage cell lines selected for their poor ability to bind EIgG rosettes. These variant cells not only formed fewer rosettes with EIgG cells than the parent line, but the rosetting was now trypsin sensitive and was completely inhibitable by monomeric IgG2a. Furthermore these cells no longer formed rosettes with erythrocytes opsonised with a cloned anti-erythrocyte IgG2b antibody. They therefore inferred the presence of two receptors; FcRI which binds cytophilic IgG2a and is trypsin sensitive, and FcRII which binds IgG2b. Both types of receptor bound immune complexes and FcRII was selectively lost in the mutant cell line. Comparable evidence for the binding of complexes to both receptors was found by Diamond et al (1978) who showed that both IgG2a and IgG2b were required to inhibit rosette formation of EIgG.

Anderson and Grey (1977, 1978) and Heusser, Anderson and Grey (1977) have also found evidence to support the view that there are two distinct receptors using myeloma proteins and macrophage cell

lines. In normal mouse macrophages and two cell lines, strong cytophilic binding of monomeric IgG2a and to a lesser extent IgG2b, but not IgG1 or IgG3 was found. However aggregated IgG1, IgG2a and IgG2b but not IgG3 all bound to a similar degree and were each inhibited by aggregated IgG in a subclass unrestricted fashion. However when monomeric inhibitors were used, IgG2b preferentially inhibited binding of aggregated proteins of all three subclasses 10 times more efficiently than monomeric IgG2a and IgG1. Conversely binding of monomeric IgG2b could be inhibited by aggregated IgG of any class but only by monomeric IgG of subclass 2b. Binding of aggregated IgG2b was increased up to threefold with increasing molecular weight whereas binding of aggregated IgG1 and IgG2a was unaffected by size. Studies of other cell lines revealed variants which showed no binding of monomeric IgG1, 2a, 2b or 3, normal binding of aggregated IgG1 and 2b but reduced binding of aggregated IgG2a. They therefore postulate two receptors - one which binds IgG2a (monomeric and aggregated) and another which binds aggregated IgG2b strongly and preferentially, monomeric IgG2b weakly but will also bind aggregated IgG1 and 2a. These findings are essentially in agreement with those of Unkeless and others described above.

In addition to binding subclasses of IgG it has also been shown that human monocytes (Joseph et al 1978) and rat macrophages (Capron et al 1975) carry an Fc receptor for IgE which can mediate binding and killing of schistosomula. Others (Dessaint et al 1979) have shown that aggregated IgE binds to rat macrophages and causes release of β-glucuronidase. Melewicz et al (1981) have further

characterised the receptor and found that 20% of human monocytes and 50 to 90% of rat macrophages formed rosettes with IgE sensitised erythrocytes; the receptor mediated antibody dependent cytotoxicity (ADCC) of IgE sensitised cells and was class specific. After denaturation by heating at 56°C or mild reduction and alkylation of IgE the receptor no longer bound IgE.

Some studies have been performed on the structural requirement for binding of the Fc fragment. It seems that the intact covalently bound Fc fragment is essential for binding and requires the presence of the CH2 domain (Alexander et al 1976, Diamond et al 1979, Dorrington and Klein 1982).

Isolation of Fc receptors

Attempts to isolate the two macrophage receptors (FcRI & FcRII) have resulted in varying degrees of success. Some studies have isolated two distinct FcR-like binding proteins with varying loss of receptor specificity (Anderson & Grey 1977, 1978, Anderson 1980, Lane et al 1980, Lane & Cooper 1982, Unkeless 1979, Mellman 1980, Kulczyckie et al 1980) while others have failed to isolate distinct subclass receptors (Schneider et al 1981). The molecular weight for these proteins has varied between 35,000 and 120,000 daltons.

Using affinity chromatography, Anderson and Grey (1977, 1978) did not achieve complete purifications but isolated two fractions containing receptor activities which preferentially bound to either aggregated IgG or monomeric IgG2a. The physicochemical properties of the receptor activities were also different. On sucrose gradients, the IgG2a receptor (FcRI) activity had a sedimentation coefficient of 4S while that of the aggregated IgG receptor (FcRII) was 20S i.e. of the same size as high density lipoprotein. This suggested that the 20S "FcRII-like" activity might be due to a lipoprotein molecule which had aggregated after removal of the detergent used to solubilise the membrane. In support of this, the FcRII activity was found to be destroyed by phospholipase C, but FcRI was not. Other studies on the activity of the solubilised membrane Fc receptor have shown that after destruction of its activity with phospholipase C the activity can be reconstituted by the addition of liposomes consisting of either

phosphatidylethanolamine or phosphatidylinositol (Anderson 1980). The lack of trypsin sensitivity of FcRII in the intact cell may therefore be due its hydrophobic lipid environment. Both isolated receptor activities are trypsin sensitive confirming that both contain protein.Suzuki et al (1982) have provided further evidence that the IgG2b receptor is "lipophilic". Using lysates from a murine macrophage cell line they isolated two proteins by affinity chromatography on columns coated with either aggregated IgG or with an analogue of phosphatidyl choline. After further purification on Sephadex G100 and isoelectric focussing they found that the lipophilic protein bound IgG2b not IgG2a whereas aggregated IgG bound IgG2a and not IgG2b.

Lane et al (1980) and Lane and Cooper (1982) have used aggregated IgG2a, IgG2b or IgG1 bound to sepharose to separate Fc receptors from thioglycollate mouse peritoneal macrophages or macrophage cell lines. Small amounts were used to avoid the problem of copurification of different receptors (Schneider et al 1981). Lane et al (1980) isolated two receptors from thioglycollate macrophages; one protein had FcRI-like activity and a molecular weight of 67,000 and the second FcRII-like protein, which bound IgG2b or IgG1, had a molecular weight of 54,000. Prior treatment of the macrophages with trypsin abrogated the isolation of FcRI activity but not FcRII. Proteins of slightly different molecular weight were obtained using the same technique on macrophage cell lines J774 and P388D. They also separated FcR activity from cell lines using affinity columns bound with a rat monoclonal anti-FcRII

antibody (Unkeless 1979). With this method a protein of molecular weight 65,000 and a lesser band of 47,000 were obtained. The isolates were trypsin insensitive but the specificity of the putative FcRII preparation was not tested.

These latter results are in agreement with those of Mellman and Unkeless (1980) who also used the rat monoclonal antibody as an affinity reagent. They obtained 60,000 and 47,000 dalton glycoproteins which bound to Con A and were neuraminidase sensitive. However after isolation there was loss of subclass specificity and although the proteins did not bind to IgG3 they did bind IgG1, IgG2a and IgG2b.

Kulczyckie et al (1980), using Sepharose affinity columns have isolated an FcR-like protein from rabbit alveolar macrophages. The molecular weight on PAGE varied with the gel; using 5.6% acrylamide gel it was 50,000-70,000 while in 9% gel it was 35,000-55,000. Their observations illustrate the difficulty of making valid comparisons between different sets of reported results where different methods have been used.

At the present time it is still not clear whether the putative FcRI and FcRII receptors are distinct or are one gene product which has been modified by glycosylation or cleavage on the cell membrane.

Attempts to isolate a human Fc receptor protein are still at an early stage. There is one report (Anderson 1982) describing the isolation of Fc receptor protein from the human macrophage cell line U937 which obtained two protein bands of 72,000 and 40,000;

the 72,000 dalton protein showed FcR binding for human IgG1 but not IgG2.

Numbers of Fc receptors

The approximate number of Fc receptors in mononuclear phagocytes is around 10^5 to 10^6 and varies between species and according to whether the cells are stimulated or not. A recent comparative study showed that guinea pig peritoneal macrophages have 50x more Fc receptors (10^6/cell) than neutrophil polymorphs ($2x10^4$/cell) and that the latter bind only guinea pig IgG2 (Coupland & Leslie 1983). The association constants in general are higher for polymeric than for monomeric IgG. Typical data is illustrated in Table 6 (p108).

Functional significance of subclass receptors

The functional significance of different receptors for mediating binding of different classes and subclasses of cytophilic antibody and aggregated or complexed antibody is not yet clear.

It is possible that cytophilic receptors (FcRI) may be important in mediating antibody dependent cytotoxicity whereas FcRII may be more relevant to phagocytosis. Although Haskill and Fett (1976) found that macrophage ADCC of tumour cells was blocked by IgG2a this was not found by Walker (1977) who showed that ADCC was blocked by IgG2b and phagocytosis was mediated via IgG2a. The latter seems unlikely however in view of the selectivity of the IgG2b receptor for complexed antibody. Others (Ralph et al 1980) found that all subclasses of murine IgG mediate phagocytosis and ADCC. The observations of Nitta & Suzuki (1982a, 1982b) and Suzuki et al (1982) however, strongly support the concept that the IgG2a

IgG2a (FcRI) and IgG2b (FcRII) receptors mediate different effects. In addition to the lipophilic characteristics of the IgG2b receptor, they have also shown that the IgG2a receptor (FcRI) is directly linked to adenyl cyclase and ligand binding causes a prompt rise in cAMP. The isolated IgG2b receptor however has PLA2 activity and ligand binding triggers arachidonic acid and PGE synthesis. In the intact cell the rise in PGE triggers a delayed rise in cAMP via PGE receptor stimulation.

Leijh et al (1979), using an assay of monocyte bacterial killing which is independent of the phagocytic phase, have shown that the presence of extracellular cytophilic binding of IgG1, IgG3 and C3b enhances intracellular killing; this may reflect stimulation of respiratory burst activity. The possible role of the Fc receptor for IgE in killing of schistosomula has already been mentioned.

TABLE 6 Number & affinity of Fc receptors on mononuclear phagocytes

Species	IgG class	Cell type	Temp °C	Ka $x10^{-8}$	Reference
Man	1	Monocyte	20	1.0	Alexander
	3	"	"	0.8	et al
	4	"	"	0.4	(1978)
Mouse	2a	P388D	"	0.7	Unkeless &
	2b	Per.mac.	4	0.1	Eisen (1975)

Species	IgG class	Cell type	Activ-ated	No. of receptors	Reference
Man	1	Monocyte	no	$1.6x10^6$	Alexander
	3	"	no	$3.4x10^6$	et al
	4	"	no	$2.1x10^6$	(1978)
Mouse	2a	Per.mac	no	$1.1x10^5$	Unkeless &
	2b	"	yes	$4.4x10^5$	Eisen (1975)
	2a	"	no	$0.5x10^5$	
	2b	"	yes	$2.9x10^5$	

Biochemistry and pharmacology

The differential sensitivity of Fc receptors to proteases and lipase has been discussed. In addition the activity of the Fc receptor also depends on the presence of functional sulphydryl groups, which are sensitive to iodoacetamide and p-chloromercuribenzoate (Howard and Benacerraf 1961), and also on carbohydrate components. ^{14}C-glucosamine is incorporated into the receptor in vivo (Kulczycki et al 1980) and the receptor is sensitive to neuraminidase (Mellman and Unkeless 1980).

Prostaglandins E2, F1, and F2α have variable effects, tending to cause enhancement of phagocytosis at physiological concentrations and inhibition at higher levels (Razin et al 1978). Levamisole has an enhancing affect on monocyte receptor function probably by triggering cholinergic receptors and a rise in cGMP (Schmidt and Douglas 1976).

The effect of corticosteroids on mononuclear phagocyte receptors has been studied in different ways with different results depending on the techniques used. Early studies (Kaplan and Jandl 1961) on rat in vivo "RES" clearance of serum sensitised erythrocytes (opsonin not specified) demonstrated reduced hepatic but not splenic sequestration after 20mg cortisone/kg for six days. The authors emphasise the potential effects of changes in blood flow on organ sequestration. Mollison et al (1962) in a study of human volunteers found little evidence that a six day course of 100mg cortisone/day had any effect on clearance of IgG sensitised

erythrocytes. Atkinson et al (1973) were able to show steroid induced reduction of clearance of both IgM and IgG sensitised erythrocytes, but the dose of cortisone was so great (20 to 100mg/kg) that their observations bear little resemblance to pharmacological therapy in humans.

In general, studies in vitro have shown effects on Fc and C3 receptor expression only with relatively large doses of steroid. Rinehardt et al (1974) showed that two hours preincubation with 16ug/ml of hydrocortisone succinate had no effect on phagocytosis of complement coated cryptococci or binding of IgG sensitised erythrocytes (EA) while high doses (120ug/ml) caused only slight reduction of complement mediated phagocytosis. Kurlander (1981) showed that 10^{-6} to 10^{-3} M hydrocortisone had no immediate effect on Fc or C3 receptor expression by monocytes, but after two days in culture with hydrocortisone, enhanced Fc and C3 receptor expression was seen. Schreiber et al (1975) found depression of Fc and C3 receptor rosetting but used vast doses (300ug/ml or 10^{-4} to 10^{-3} M) of corticosteroid in cholesterol-phospholipid liposomes to achieve the effect.

Two recent studies illustrate best the potential complexity of the effect of steroids. Rinehardt et al (1975) studied blood monocyte function in vitro before and after administration of 50mg prednisolone 12 hourly for three days to normal volunteers. After transient initial monocytopenia, a sustained doubling in the monocyte count was seen. Phagocytosis of complement opsonised cryptococci was enhanced three-fold after therapy and no impairment

was found in hexose monophosphate shunt activity. Furthermore, chemotaxis was significantly increased. The only function to be depressed was killing of staphylococci and candida, which suggests that lysosomal function may have been impaired. More recently Ralph et al (1978), using a macrophage cell line, showed that 10^{-4}M hydrocortisone had no effect on latex or Fc mediated phagocytosis or ADCC. However hydrocortisone did block activation of the cells by lipopolysaccharide (LPS) or PPD and reversed the growth inhibitory effect of LPS. Other studies have also shown that while glucocorticosteroids block the egress of monocytes from the marrow and their emigration to inflamed tissues in the tissues, they do not block proliferation or maturation (van Furth 1970).

These observations can be summarised as follows. Pharmacological doses of corticosteroids have no direct inhibitory effect on Fc or complement receptor expression. Studies of the in vivo effect of corticosteroids on monocyte function must be interpreted in the knowledge that profound changes in monocyte traffic are occurring and that the egress of more mature monocytes from the circulation may be inhibited. This is highlighted by the very marked monocytosis seen in human studies. Studies of steroids on in vivo "RES" clearance are hard to interpret in view of the potentially multiple sites of action of steroids and in particular on organ blood flow, and studies in animals using very large doses of steroid must be viewed with caution.

Modulation of mononuclear phagocyte receptors by immune complexes

To examine the modulating effect of immune complexes on mononuclear phagocyte receptor expression, some workers have used soluble immune complexes while others have used immobilised immune complexes.

Several studies have shown that plating macrophages onto IgG coated glass causes marked loss of Fc receptor rosetting and phagocytic capability (Rabinovitch et al 1975; Douglas 1976; Kaplan et al 1978; Ragsdale and Arend 1980; Arend and Massoni 1981; Michl et al 1979). Some studies (Ragsdale and Arend 1980; Michl et al 1979) suggest that, at least with activated macrophages, Fc receptor phagocytic function is more sensitive to this procedure than Fc receptor rosetting. The studies of Michl et al (1979) suggest that the loss of phagocytic function reflects selective loss of the trypsin resistant, IgG2b-Fc receptor (FcRII) leaving residual FcRI receptors available for rosetting. All these studies agree that loss of FcR activity is selective and neither affects complement receptor rosetting by resident cells nor complement mediated phagocytosis by thioglycollate elicited cells.

Some studies have also looked at the effect of the presence of complement in the immobilised complexes. Two studies, show that complement binding to the immune complexes has no modulatory effect on complement receptor activity in either resting or activated macrophages (Kaplan et al 1978; Ragsdale and Arend 1980). One study however (Arend and Massoni 1981), using human monocytes, showed a

minimal reduction in complement receptor rosetting when fresh monocytes were plated onto complement-fixed immune complexes, but that the effect could be enhanced by prior culture of the cells for several days in serum. In another study (Michl et al 1979), where murine macrophages were plated onto complement-fixed immune complexes, no modulating effect was found if resident peritoneal macrophages were used, whereas thioglycollate elicited macrophages lost both complement receptor rosetting and phagocytic function.

Two studies with soluble immune complexes have also produced conflicting results with respect to complement receptor modulation but have both demonstrated Fc receptor modulation. Kavai et al (1981) used soluble complexes of BSA or ovalbumin with IgG prepared in various ratios and with antibody of varying avidity. The experiments were also performed after complement fixation by the immune complexes. Exposure of human monocytes to Fc bearing immune complexes caused inhibition of Fc rosetting and Fc mediated phagocytosis of erythrocytes but had no effect on complement receptor mediated phagocytosis of C3 coated yeast. The effect was most marked when antibody of high rather than low avidity was used and when complexes were prepared at antibody-antigen equivalence or slight antibody excess. After exposure of cells to complexes which had fixed complement, inhibition of phagocytosis of C3b coated yeast was seen. Griffin (1980) on the other hand found that IgG containing complexes with or without complement blocked Fc receptor mediated phagocytosis but did not inhibit complement mediated phagocytosis.

Ragsdale and Arend (1980) investigated the possible mechanism of Fc receptor modulation and found evidence to suggest that it is mediated by a rise in intracellular cAMP. The loss of Fc receptor activity on binding to an immune complex coated surface could be abrogated by incubation with 2-deoxyglucose (2DG) which blocks synthesis of ATP and prevents the rise in cAMP. The effect of 2-DG could be reversed by the addition of exogenous ATP and similar results were obtained with other metabolic inhibitors.

SECRETORY PRODUCTS OF MONONUCLEAR PHAGOCYTES

Introduction

The mononuclear phagocyte secretes a wide range of products (Table 7, p116) which participate in inflammation, immunity and the regulation of connective tissue cells and their matrix. In rheumatic disease, the generation of prostaglandins and the interaction of macrophages with connective tissue have assumed considerable importance and a brief review of these areas is therefore relevant to this thesis.

TABLE 7 Secretory products of mononuclear phagocytes
(Nathan et al 1980)

ENZYMES
Lysozyme
Neutral proteases
 collagenase
 elastase
 angiotensin convertase
 plasminogen activator
Acid hydrolases
 proteases
 lipases
 deoxyribonucleases
 phosphatases
 glycosidases
 sulphatases
Arginase

COMPLEMENT
C1, C2, C4,
C3, C5
Factor B, D
Properdin
C3bINA
bIH

ENZYME INHIBITORS
plasmin inhibitors
alpha2 macroglobulin

NUCLEOSIDES etc
thymidine
uracil
uric acid

REACTIVE OXYGEN METABOLITES
superoxide
hydrogen peroxide
hydroxyl radical
singlet oxygen

BIOACTIVE LIPIDS
PGE2, 6-ketoPGF1a,
thromboxane
leukotriene
HETE &SRSA
platelet activating factors

FACTORS REGULATING PROTEIN
SYNTHESIS IN OTHER CELLS
Serum amyloid A
Haptoglobin
Collagenase

FACTORS PROMOTING REPLICATION
of lymphocytes (LAF)(IL1)
 myeloid precursors (CSF)
 erythroid precursors
 fibroblasts
 microvasculature

FACTORS INHIBITING REPLICATION
of lymphocytes
 tumour cells
 viruses (interferon)
 Listeria monocytogenes

Prostaglandin secretion by mononuclear phagocytes

Macrophages from a wide variety of sources have a very high arachidonic acid content and produce significantly greater amounts of E type prostaglandins (Kurland and Bockman 1978, Goodwin et al 1977a, Scott et al 1980, Stossel et al 1974) and leukotrienes (Scott et al 1980, Rouzer et al 1980b, Valone et al 1980) than other leucocytes. There are a wide range of stimuli which activate macrophage prostaglandin synthesis and include IgG-Fc (Rouzer et al 1980a) and IgE-Fc (Rouzer et al 1982) receptor stimulation, lymphokines (Gordon et al 1976), endotoxin (Ogmundsdottir and Weir 1979, Kurland and Bockman 1978), complement activation products (Rutherford and Schenkein 1983), C. parvum (Ogmundsdottir and Weir 1979), zymosan (Humes et al 1980) and phorbol myristate acetate (PMA)(Bonney et al 1980b).

Although phagocytic stimuli including immune complexes stimulate prostaglandin secretion and synthesis, the latter can be clearly separated functionally from the process of endocytosis. Scott et al (1980) have shown that although the rate of PG secretion is proportional to the concentration of a phagocytic stimulus, inhibition of interiorisation of IgG opsonised particles by cytochalasin D or inhibition of phagosome-lysosome fusion with dextran has no effect on PG release. Conversely, uptake of unopsonised polystyrene beads is not accompanied by PG release while "frustrated phagocytosis" of large IgG coated sephadex beads again causes PG release emphasising the importance of membrane receptor-ligand interactions.

PG secretion has also been shown to be distinct from the enhanced cytotoxicity seen in activated macrophages. In vivo activation of peritoneal exudate macrophages (PEM) by injection of either C. parvum, pyran or glucan results in increased tumoricidal capability 4 days after injection. However, of these three stimuli, only C. parvum is associated with increased PGE2 production in PEM and spleen cells (Barlin et al 1981). Thioglycollate elicited macrophages also demonstrate enhanced secretory and pinocytotic activity (Cohn & Benson 1965, Werb and Gordon 1975, Werb and Gordon 1975a, Unkeless et al 1974) but, unlike macrophages activated with immune stimuli such as C. parvum, they exhibit reduced resting rates of PGE2 secretion and reduced rates of stimulated PG release to immune complexes, PMA or zymosan compared with resident macrophages (Bonney et al 1979, 1980b, 1981, Humes et al 1980). It has been suggested (Bonney et al 1981) that this may be due to inactivation of the PG cyclooxygenase and PGI2 synthetase by toxic oxygen species (Egan et al 1976) however it may simply reflect the fact that thioglycollate macrophages are recently recruited from the blood stream and represent a different population of cells. It is clear however that while a range of functional activities are associated with changes in PG synthesis they may not be directly linked.

Although prostaglandin production can be clearly dissociated from other apparently synchronous events there may none the less be a common initiating pathway for increased arachidonic acid synthesis and activation of cytoplasmic effector mechanisms.

Activation of macrophages whether by nonimmune (eg endotoxin) or immune lymphokine dependent mechanisms results in increased turnover of membrane phosphatidyl choline (Ogmundsdottir and Weir 1979). Hydrolysis of the latter by phospholipase A2 generates arachidonic acid but is also accompanied by an influx of divalent calcium which initiates cGMP synthesis and the cytoplasmic events associated with cellular activation. Paradoxically, PGE2 may stimulate opposing effects in macrophages by activating adenyl cyclase and the synthesis of cAMP (Gemsa et al 1979), and the function of PGE2 may therefore be to act as an endogenous modulator.

As discussed already, studies on murine cell lines have provided strong evidence that the IgG2b-Fc receptor (FcRII) has phospholipase A2 activity which is enhanced by ligand binding thus providing a direct link to arachidonic acid synthesis (Nitta & Suzuki 1982a, 1982b). Furthermore, there is also direct evidence to support the hypothesis that Fc receptor-ligand coupling directly stimulates cationic influx into the phagocyte. It has been shown in intact cells that membrane depolarisation secondary to sodium influx follows rapidly after Fc-ligand coupling and this is followed by ouabain sensitive hyperpolarisation. Experiments with isolated cell vesicles and synthetic proteoliposomes incorporating purified IgG2b-Fc receptor confirm that the isolated receptor behaves as a ligand dependent ionophore triggering changes in membrane permeability and selective cationic fluxes (Young et al 1983).

The importance of prostaglandin production by macrophages lies in their regulatory effects on other cell populations as well as on the macrophage itself. For example, Wahl et al (1974) showed that the stimulation of guinea pig macrophages by endotoxin to produce collagenase could be abrogated by indomethacin and that the blocked cultures could be reactivated by the addition of PGE2. PGs also have profound effects on lymphokine generation by lymphocytes and on antibody synthesis. In a variety of model systems it is clear that the macrophage can suppress lymphocyte function via PGE2 secretion and that this can be blocked by removal of the macrophage or addition of indomethacin (Ellner 1981). Gordon et al (1976) demonstrated that lymphokines from antigen sensitised lymphocytes stimulated PGE2 release from macrophages and that PGE2 had a "feed-back" inhibitory effect on lymphokine (MIF) secretion. The feed-back inhibition could be abrogated by indomethacin. In animal models it has also been demonstrated that PGE2 production by macrophages causes suppression of lymphocyte responses. Injection of C. parvum, pyran or glucan, as mentioned above, causes macrophage activation and enhanced cytotoxicity but only in C. parvum injected animals is this associated with increased PGE2 synthesis by macrophages and spleen cells (Barlin et al 1981, Grimm et al 1978). In parallel with the rise in PG production the proliferative response of spleen cells to Con A is suppressed and, at least initially, this is reversed by indomethacin or removal of macrophages. Later, by eight days after injection, the suppression persists but is no longer indomethacin sensitive and may be

attributable to hydrogen peroxide mediated suppression (Metzgar et al 1980).

There is also evidence to suggest that products of the lipoxygenase pathway may have enhancing effects on lymphocyte proliferative responses. Kelly et al (1979) demonstrated that lipoxygenase inhibition caused reduced lymphocyte transformation to PHA and more recent reports (Dinarello et al 1983) have shown that the enhancement of thymocyte proliferation by macrophage derived interleukin 1 (leucocyte pyrogen or LAF) is inhibited by BW755C, a selective lipoxygenase inhibitor.

Depressed lymphocyte proliferation in vitro and immune anergy in vivo has been attributed in anumber of diseases in man and animals to excessive PG production by macrophages. The diminished response of peripheral blood lymphocytes to PHA in Hodgkins disease is reversed by indomethacin or removal of adherent cells (Goodwin et al 1977b). Similarly in rheumatoid arthritis, peripheral blood monocytes synthesise considerably higher levels of PGE2, TXB2 and 6-keto-PGF2a compared with controls and is associated with depression of con A induced lymphocyte proliferation. However although the effect is reversed by reducing the number of monocytes present it is not reversed by indomethacin suggesting that other mechanisms may also play a part (Seitz 1982).

Interactions of mononuclear phagocytes with connective tissue

Introduction

Although mononuclear phagocytes play a prominent role in chronic inflammatory responses and secrete a range of potentially destructive neutral proteinases and acid hydrolases, the evidence that they play a direct role in degradation of the extracellular matrix is of necessity largely indirect.

The rheumatoid joint often shows extensive evidence of cartilage and soft tissue destruction which is associated with the presence of exuberant pannus containing macrophages, lymphocytes and lymphoid follicles and, intermittently, a neutrophil infiltrate exudate in the synovial fluid. In addition, a wide variety of potential stimuli to macrophage secretion are found in the joint which include immune complexes, complement activation products and lymphokines. However while a variety of cellular and humoral mediators of connective tissue damage are clearly present the relative contribution of the macrophage and its products is still poorly understood. Interest in the macrophage has focussed on two different facets of its function. Firstly the secretion of enzymes which might directly degrade connective tissue, and secondly the secretion by macrophages of intercellular hormones which may modulate the activities of connective tissue cells and thus indirectly influence the connective tissue matrix.

Mononuclear phagocyte derived enzymes

The mononuclear phagocyte has a rich content of lysosomal acid hydrolases (Cohn and Wiener 1963) and is capable of active secretion of neutral proteinases including plasminogen activator (Gordon et al 1974), elastase (Werb and Gordon 1975b) and collagenase (Werb and Gordon 1975a). When stimulated, for example by immune complexes, they also exhibit prolonged secretion of lysosomal enzymes (Cardella et al 1974). It is likely that because of their pH optima that the neutral proteinases will play a dominant role in initial extracellular degradation while lysosomal enzymes will be predominantly engaged in the completion of digestion within the phagolysosome following endocytosis of partially degraded connective tissue (Werb et al 1980a).

A variety of factors influence the capacity of macrophages to secrete neutral proteinases and include the immunological and inflammatory status of the experimental animal or system and the presence of added stimuli in vitro. For example peritoneal macrophages elicited with an inflammatory stimulus such as thioglycollate secrete greater amounts of plasminogen activator (Unkeless et al 1974), elastase (Werb and Gordon 1975b) and collagenase (Werb and Gordon 1975a) than resident macrophages. Resident macrophages on the other hand can be induced to secrete these enzymes by feeding them with non-degradable phagocytosable particles such as latex beads (Werb and Gordon 1975a,1975b).

A comparison of the relative activities of various enzymes in resident and inflammatory or "elicited" macrophages is shown in Table 8.

TABLE 8 Relative activities of resident and inflammatory macrophages (thioglycollate elicited)
Cohn (1978)

Enzyme	Ratio of activity (inflam/resident)
5' nucleotidase	0.01
lysozyme	0.9
collagenase	15
elastase	38
plasminogen activator	800
cathepsin D	3
b-glucuronidase	5
acid phosphatase	14

The immunological status of the animal is also important. Peritoneal macrophages from mice injected with endotoxin secrete very little plasminogen activator spontaneously in vitro but show greatly enhanced rates of plasminogen activator release to a subsequent phagocytic stimulus such as latex particles (Gordon et al 1974) Perhaps of greater importance to immunological disease such as rheumatoid arthritis is the observation that T-cell lymphokines cause enhanced plasminogen activator secretion from unstimulated resting macrophages. Similarly, enhanced plasminogen activator release is seen in macrophages from animals receiving secondary antigenic challenge with BCG or T. cruzei and suggests an important role in delayed hypersensitivity (Gordon et al 1978).

Werb and others (Jones and Werb 1980, Werb et al 1980b, 1980a) have studied the capacity of both macrophages and purified

macrophage proteinases and acid hydrolases to degrade connective tissue matrix synthesised in vitro by smooth muscle and endothelial cells. Smooth muscle cells synthesise a glycoprotein/elastin/collagen matrix while the latter produce glycoprotein and collagen alone. Purified macrophage plasminogen activator in the presence of added plasminogen generates plasmin which degrades a large portion of the glycoprotein. This step was shown to be important as it facilitated the access of elastase to its major substrate elastin and in the absence of plasmin, elastin degradation proceeded very slowly. Elastase also contributed to some extent to glycoprotein breakdown while collagenase degraded only collagen. Plasmin also plays a role in the activation of collagenase which is secreted in latent form (Werb et al 1977). Macrophage conditioned medium was much less efficient than purified enzyme in degrading matrix and probably reflects the fact that over 90% of these enzymes are secreted in latent form. It is also important to note that there were differences in the capacity of macrophages elicited with different stimuli to degrade the matrix. Resting cells caused minimal degradation of glycoprotein only; endotoxin or pyran copolymer elicited macrophages secreted plasminogen activator only and therefore caused glycoprotein but not elastin degradation, while thioglycollate or periodate elicited macrophages secreted plasminogen activator, elastase and collagenase. The soluble fragments created by the neutral proteinase digestion were relatively large and complete degradation to amino acids and oligopeptides required the action of cathepsins

at acid pH. In vivo this almost certainly occurs intracellularly or in the pericellular space where the local pH may be sufficiently low. Morphological studies of live macrophages in this system showed that matrix breakdown occurred mainly in the immediate vicinity of macrophages, emphasising the importance of pericellular events where local enzyme concentrations will be highest, inhibitors are likely to be saturated and other factors such as free radical damage may play a role in the initial degradative attack. Membrane bound enzymes may also play an important role in this regard (Lane et al 1980). In RA synovium, immunofluorescence studies have confirmed that active collagenase is present at the pannus-cartilage junction but not elsewhere in the synovium (Woolley et al 1977).

Studies of the characteristics of macrophage elastase revealed that it is a metalloproteinase with a number of interesting properties (Banda and Werb 1979, 1980a, 1980b). The enzyme is secreted in latent form by the macrophage but it is not known how it becomes activated in vivo. Apart from elastin it also degrades other connective tissues including laminin (a basement membrane component), microfibrillin (a glycoprotein associated with elastin) and fibronectin as well as both fibrin and fibrinogen. Two other substrates suggest that the enzyme may have important regulatory properties in inflammation. It was found to attack the heavy chain of IgG2a but not IgM, A or G2b and in view of the specificity of IgG2a for the FcRI receptor this could reflect an autoregulatory role in inflammation. Additionally, it is not inhibited by

alphal-proteinase inhibitor and in fact degrades this inhibitor and prevents it from inhibiting granulocyte elastase (Banda et al 1981). The latter observation suggests that it may play an important role in emphysema.

Mononuclear phagocyte derived inter-cellular hormones

An alternative view of connective tissue matrix breakdown has been proposed by Krane and others (reviewed by Krane 1981) and Fell and others (reviewed by Dingle 1981) who have found strong evidence to suggest that the macrophage and its constituent enzymes is not directly responsible for matrix destruction. This model proposes that release of intercellular hormones or "cytokines" by monocytes stimulates other synovial, fibroblast-like cells to generate collagenase and mediate tissue degradation.

Early studies of rheumatoid synovium had shown that active collagenase could be recovered from the synovial fluid (Harris et al 1969; Harris et al 1970), that cartilage and soft tissue destruction was related anatomically to sites of synovial pannus formation (Kulka et al 1955) and probably that synovial derived collagenase and neutral proteases were involved in tissue damage (Harris and Krane 1974).

Tissue culture studies of synovial dispersates revealed that a population of large stellate adherent cells were present which contained abundant collagenase and PGE2, while the macrophages present had little or no detectable collagenase (Dayer et al 1976; Wooley et al 1978; Wooley et al 1979). Furthermore, on longer culture, loss of the mononuclear population was associated with diminution but not loss of collagenase synthesis by the stellate cells which suggested that the mononuclear cells were in some way indirectly stimulating collagenase synthesis. Further studies

confirmed that although monocytes and lymphocytes were not producing collagenase (Dayer et al 1980) the inclusion of monocytes or monocyte conditioned medium caused a considerable increase in collagenase and PGE2 production by stellate adherent cells (Dayer et al 1980; Dayer et al 1977; Dayer et al 1979; Dayer et al 1979a). Attempts to purify the mononuclear cell factor (MCF) responsible for this effect have so far revealed a single product which is partially characterised and shares biological and biochemical properties with interleukin 1 (IL1); the effect on PGE2 and collagenase release appears to be attributable to the one product (Dayer et al 1979; Dayer et al 1979a; Dayer et al 1981; Mizel et al 1981). A similar observation has been made by Postlethwaite et al (1983) who have shown that IL1 stimulates collagenase production by dermal fibroblasts.

A further level of control of collagenase production has been demonstrated in that monocyte synthesis of MCF can be modulated by lectin activated lymphocytes or their supernatant media; small numbers of T-cells enhance while large numbers inhibit MCF production suggesting suppressor-helper like effects (Krane 1981). Also of relevance to RA is the observation that Fc fragments or aggregated IgG stimulate both PG production and MCF release by macrophages and that blocking PG production with indomethacin does not affect MCF release (Passwell et al 1979; Dayer et al 1980).

The production of PGE2 by synovial cells and monocytes provides a further level of control over the system by stimulating cAMP synthesis which limits the actions of MCF. However while

exogenous PGE2 causes a sharp rise in cAMP in cultures of synovial cells alone, the effect is not nearly as marked on cocultures of synovial cells with monocytes. The suggested explanation for this phenomenon is that endogenous PGE2 synthesis in the monocyte-synovial cell system causes "down-regulation" of PGE2 receptors. This can be overcome, and the response to exogenous PGE2 restored, by including indomethacin to eliminate endogenous PGE2 from the system (Dayer et al 1979; Goldring et al 1980). PGE2, not MCF, is solely responsible for the rise in cAMP; blocking of the PGE2 induced rise in cAMP with indomethacin "unmasks" other actions of MCF on synovial cells including a proliferative effect and enhancement of collagen and fibronectin synthesis (Dayer et al 1979).

Parallel observations using an organ culture system (Fell & Barrett 1973, Fell & Jubb 1977, Dingle et al 1975, Barrett et al 1977, Fell et al 1976) to study the effect of synovial tissue on live cartilage matrix have produced a similar picture. The ability of normal and rheumatoid synovium to degrade cartilage matrix was first shown by Hamerman et al (1967), and subsequent work has confirmed that normal synovium in culture causes cartilage degradation (reviewed by Fell 1978). The initial changes are of rapid loss of metachromatic proteoglycan followed by a slower loss of collagen. The appearance of the chondrocytes also changes and they become basophilic and divide forming groups of cells within the cartilage. Eventually these cells leave the cartilage forming a layer of fibroblast-like cells. Although these changes are maximal

when the synovium is in direct contact with the cartilage, they also occur, albeit more slowly, when the synovium and cartilage are not in direct contact. Furthermore, dead cartilage is less quickly degraded than live cartilage containing viable chondrocytes under these conditions.

These observations suggested that soluble mediators were being released from synovium which stimulated chondrocytes to degrade their own matrix and subsequently to proliferate. A number of subsequent studies have demonstrated that supernatants from cultured synovial tissue or synovial cells in monolayer contain a protein of molecular weight approximately 17,000, provisionally termed "catabolin" which mediates these effects on chondrocytes in nanogram quantities (Saklatvala and Dingle 1980). The mechanism of matrix degradation by the chondrocyte is not certain but is associated with the action of pericellular proteinase secretion (Dingle and Dingle 1980). The relationship of "catabolin" to other cytokines such as MCF and IL1 is not known although they may form a family of proteins analogous to the interferons. Furthermore it is not certain whether the synovial cell responsible for producing catabolin-like activity is the macrophage or some other cell or cells.

In conclusion, the relative role of macrophage derived proteinases and the indirect effects of the macrophage on other connective tissue cells have not been resolved, but it is likely that both play a complementary role in mediating tissue degradation.

CHAPTER 3

AETIOPATHOGENESIS OF RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

AETIOPATHOGENESIS OF RA AND SLE

Introduction

Both RA and SLE are systemic diseases characterised by a number of immunological abnormalities including autoantibody and immune complex formation (Koffler et al 1971; Zvaifler 1981; Nydegger & Lambert 1982) as well as abnormalities of cell mediated immunity (Paty et al 1975; Horwitz 1972, 1982; Hahn et al 1973).

The aetiology of both diseases is unknown but it is likely that environmental triggering agents as well as host genetic susceptibility play a role. The evidence for this view will be discussed shortly and it is perhaps pertinent to begin with a review of the role of exogenous agents in the development of one or two other examples of chronic inflammatory arthritis and autoimmune disease.

The role of infection - introduction

The microbe-host interaction is complex and a multitude of factors determine the outcome. A number of well characterised viruses and bacteria cause arthritis, vasculitis and other allergic sequelae in humans and serve to illustrate potential pathogenetic mechanisms which may be relevant to RA and SLE. For example, recovery from hepatitis B virus infection is associated with a phase of intense production of immune complexes containing viral antigen shed from infected cells and is manifested clinically by serum sickness like symptoms with arthralgia, arthritis and urticaria (Malawista & Steere 1981). Similar events may occur during meningococcal infection (Greenwood & Whittle 1976). Chronic hepatitis B virus infection has also been implicated in a significant proportion of patients with systemic vasculitis (Sergent et al 1976). Other organisms such as vaccinia and rubella which are arthrotropic viruses trigger arthritis by direct infection of joints. Rubella arthritis may relapse intermittently over periods of several months and although the virus is recoverable from joint fluid during a relapse (Grahame et al 1981) it is undetectable during remissions. "Reactive arthritis" which may be triggered by certain enteric organisms such as Shigellae, Yersiniae and Salmonellae occurs predominantly in genetically predisposed individuals carrying the HLA-B27 tissue type (Ford et al 1983), but despite this clear association the pathogenetic mechanism is obscure.

Lyme disease is another interesting example of a host-parasite

interaction which may be associated with chronic erosive arthritis. Lyme disease which was first described in Connecticut in 1975, occurs during summer months and begins with a unique skin lesion - erythema chronicum migrans - which may be accompanied by headache, myalgia, fever, arthalgia or lymphadenopathy (Steere et al 1983). A proportion of patients subsequently develop neurological and cardiac complications and recurrent attacks of frank arthritis which may become chronic and erosive (Steere et al 1979, 1980). The disease is associated with a number of immunological abnormalities including circulating immune complexes, and chronicity is associated with the B cell alloantigen DR2 (Steere et al 1979). The seasonal incidence and initial skin lesion suggested an arthropod vector and this led to the discovery that the disease was transmitted by a tick - Ixodes dammini. Very recently it has been shown that the infectious organism responsible for the disease is a previously unrecognised spirochaete (Steere et al 1983). The antibody response to the organism in some patients is abnormal in that they show a persistent specific IgM response and a delayed IgG response suggesting impairment of the T-helper cell dependent switch from IgM to IgG. Although the disease is distinguishable from "immune-mediated" disease such as RA, it none the less mimics a number of immunological disorders such as juvenile chronic arthritis, multiple sclerosis, Reiters syndrome and Guillaine-Barre syndrome and illustrates the importance of host HLA-DR antigens in predisposing to chronic inflammatory disease.

Role of infectious agents in SLE

Although there is a clear relation between some forms of chronic arthritis and infectious agents there is at present no definite evidence for an infectious cause of SLE in man or mouse.

Some studies of murine lupus have shown that certain "autoimmune" strains (NZB; NZB/W F1 hybrids; MRL/1) have high circulating levels of Type C retrovirus glycoprotein (gp70) (Izui et al 1978) which suggests that retrovirus might be responsible for the development of autoimmunity. However other genetic studies of NZ mice have failed to support an association between viral expression and autoimmunity and furthermore other autoimmune strains such as Swan mice do not produce xenotropic C type viral particles (Blaineau et al 1978). However it is possible that increased retrovirus antigen expression and immune complex formation, occurring as a consequence of abnormal immunoregulation, may play a role in the evolution of immune complex mediated glomerulonephritis in murine lupus (Yoshiki et al 1974). Latent Herpes viruses may similarly cause immune complex mediated glomerulonephritis in immunosuppressed humans (Schooley et al 1983). Viruses may alter the course of murine lupus in other ways. For example infection with pathogens such as Sendai virus accelerates production of autoantibody (Steinberg et al 1981) while infection with lactate dehydrogenase virus retards autoimmunity (Oldstone and Dixon 1972). Extensive searches for a retroviral cause of human SLE have so far been unsuccessful (Pincus 1982).

Serological studies of human SLE have shown increased antibody

titres to numerous RNA and DNA vuruses including parainfluenzae, measles, mumps, rubella, reovirus, polio, adenovirus, Herpes simplex, CMV and EBV but there is no evidence that any of these are aetiologically responsible (Pincus 1982). Increased antibody titres may reflect polyclonal B-cell activation, crossreacting anti-DNA antibody to common, repeating epitopes such as phosphodiester groups (Shoenfeld et al 1983), or defective cell mediated immunity allowing increased expression of latent DNA viruses and a secondary rise in humoral responses (Henle and Henle 1981). None the less, circumstantial evidence for a viral aetiology including increased alpha-interferon production (Friedman et al 1982) and cross-reactivity of SLE sera with virus specific RNA (Lerner et al 1982) is tantalising and provides a continuing impetus to research in this area.

Role of infectious agents in RA

In RA, as in SLE, there is no definitive evidence that the disease is due to an infectious agent or microbial product. Isolation of diptheroids and mycoplasmae from synovial tissue by earlier workers have not been substantiated and although mycoplasmae cause arthritis in animals (Cole & Cassel 1980) there is no convincing evidence that mycoplasmae cause RA. A search for bacterial cell wall components in RA tissue using mass spectrometry has also proved negative (Pritchard et al 1980). As in SLE, attention has focussed on the possible role of retroviruses but no evidence of infection of RA lymphocytes (Norval et al 1979, Hart et al 1979) or synovial cells (Hart et al 1980) has been found.

The observation that RA serum appeared to contain a unique immune precipitin (RAP) directed against Wil2, an EBV infected cell line (Alspaugh & Tan 1976; Alspaugh & Henle 1979), and the demonstration by immunofluorescence of an antinuclear antibody (RANA) directed against EBV infected B-cell lines (Alspaugh et al 1979) fuelled a search for an aetiological association between RA and EBV. Furthermore the tropism of EBV for B-cells and its ability to induce B-cell proliferation makes it a candidate virus for activation of "autoimmune" B-cell clones in vivo. Although it is now clear that EBV is not aetiologically related to RA, a review of recent studies in this area will illustrate some of the problems of interpretation of viral studies in patients with diseases of disordered immunity.

The tropism of EBV for B cells is determined by a specific

membrane receptor closely associated with the C3d receptor. Uptake of the virus via this receptor generally results in latent "nonproductive" symbiotic infection which "freezes" the differentiation of the host cell and gives it unlimited growth potential (Klein & Purtilo 1981); following primary infection a small number of B-lymphocytes remain latently infected for life (Depper & Zvaifler 1981). In a small proportion of cells lytic "productive" infection occurs which results in release of infective virions and cell death.

Expression of viral associated antigens after infection of B cells follows a similar pattern both in vivo and in vitro. However, antigen expression during "lytic" infection of B cells is different from that following "nonlytic" latent B cell transformation. This difference is reflected in the immune response to these two events and is partly responsible for the different patterns of immunity between normal individuals with latent infection and those with varying degrees of immunodeficiency in whom lytic "productive" infection may become more intense (Henle & Henle 1981).

Following lytic "productive" infection of B cells three antigens appear - Membrane Antigen (MA), which is expressed both on the cell membrane and on the virus envelope; Early Antigen (EA), which has two components and is either restricted to the cytoplasm (R) or diffusely located in both nucleus and cytoplasm (D); and Viral Capsid Antigen (VCA). After nonlytic blast transformation of B cells by EBV two antigens (or groups of antigens) appear - Lymphocyte Derived Membrane Antigen (LYDMA) and EB Nuclear Antigen

(EBNA). LYDMA is operationally defined using specific cytotoxic T cells and EBNA is serologically defined.

Primary EBV infection is characterised by the appearance of IgM and IgG antibodies to VCA and transient antibody to EA(D). IgG anti VCA persists and is a standard epidemiological marker for previous EBV infection, but IgM anti VCA and IgG anti EA(D) subside within weeks. Anti heterophile antibody and neutralising antibody to MA also develop during the acute phase. During convalescence, usually months after the primary infection, antibodies to EBNA appear which subsequently remain stable for years (Henle & Henle 1981). In addition to these humoral responses several cell mediated immune defence mechanisms are initiated. Natural killer cells, interferon activated killer cell activity and antibody dependent cellular cytotoxicity (ADCC) play a role in nonspecific lysis of infected cells entering the lytic phase and are therefore particularly evident during Infectious Mononucleosis (Tosato et al 1982). On the other hand proliferation of EBV transformed "nonproductive" cells is inhibited by EBV specific T cell mediated immunity directed against LYDMA (Rickinson et al 1981). Both specific and nonspecific mechanisms are probably important in maintaining a stable symbiotic state in the normal subject and prevent uncontrolled B cell proliferation.

Studies of the role of EB virus in RA have addressed the following questions: 1) Is there seroepidemiological evidence of increased exposure to EBV in RA patients? 2) Is there evidence of defective cellular immunity to EB virus in RA? 3) Is the RAP/RANA

antibody-antigen system unique to RA patients? 4) Does the RAP/RANA system represent immunity to a unique EBV encoded antigen? 5) Is there evidence of increased EB virus shedding from patients with RA?

Serological studies have shown a more or less similar frequency of anti-VCA and anti-EBNA in RA patients and controls and furthermore about five percent of RA patients have no serological evidence of primary EBV infection at all (Venables et al 1980, 1981; Elson et al 1979; Phillips et al 1973; Silverman et al 1981). This must imply that an aetiological role for EBV in all RA patients is very unlikely. Increased titres to VCA and EA, especially EA(D), have been found in several studies (Alspaugh et al 1981; Ferrel et al 1981), but as noted above, these changes almost certainly reflect minor impairment of cellular immunity and a secondary increase in numbers of cells entering the lytic phase.

On the other hand evidence to support the view that there is defective EBV specific cellular immunity in RA is substantial. The ability of various concentrations of T-cells to induce regression of spontaneous proliferation of B cells in vitro is used as a quantitative measure of EBV specific T cell immunity (Rickinson et al 1981) and has been used to demonstrate defective EBV specific cellular immunity in RA patients (Tosato et al 1981). It has been shown that peripheral blood mononuclear cells from RA patients have an increased tendency to spontaneous B cell proliferation in vitro (Slaughter et al 1978; Bardwick et al 1980) and this almost certainly reflects diminished T cell control rather than an

increased proliferative capacity of the B cell per se (Depper & Zvaifler 1981). Although this defect is not restricted to RA patients, and is found in chronic infectious mononucleosis and Duncans disease (Masucci et al 1981), the evidence at present suggests that the defect in RA may be restricted to the T cell specific EBV response whereas in other diseases the cellular defect may be more generalised and involves NK cell and interferon induced K cell activity. At present there is insufficient data to be certain that a restricted defect of EBV specific T cell immunity is unique to RA.

Other workers have examined whether the RAP/RANA system (Venables et al 1981) is unique to RA. The first description of RAP suggested that it was unique to RA (Alspaugh & Tan 1976; Alspaugh & Henle 1979), but it is now clear from subsequent studies that RAP and RANA are frequently found in control subjects (Venables et al 1980, 1981; Catalano et al 1980; Ng et al 1980).

Some studies have asked whether the RAP/RANA system is unique to EB virus antigens and whether anti-RANA is found in patients with no serological evidence of primary EB virus infection. Although one study (Catalano et al 1980) found concordance between anti-VCA and RANA, others (Venables et al 1980, 1981; Ng et al 1980) have identified a small but significant number of patients who have no serological evidence of primary EB virus infection but do have RANA. Conversely some RA patients with anti-EBNA do not have anti-RANA (Silverman & Schumacher 1981). Similar observations have been made by Alspaugh et al (1979) who found a lack of

concordance between anti-RANA and anti-EBNA in RA patients, normal controls and patients with Burkitts lymphoma, and also showed that anti-EBNA and anti-RANA gave different patterns of immunofluorescent nuclear staining. Furthermore Venables et al (1981) demonstrated by adsorption studies that at least a part of anti RANA activity could be removed from high titre RA sera using non EB virus infected cell lines which therefore suggests that RANA may not be a singlet antibody-antigen system, and may contain non EB virus antigens as well.

An increase in EBV shedding from the throat might be expected if there is impairment of immunity to the virus, but this phenomenon has not been detected in RA patients (Depper et al 1982). However any defect of specific cellular immunity may be more than adequately compensated for by nonspecific cellular mechanisms and increased levels of antibody and therefore make recovery of virus difficult. Increased viral shedding from the pharynx, as well as spontaneous polyclonal B-cell lymphoproliferation, is however usually only seen in severely immunosuppressed patients such as those receiving organ transplants (Hanto et al 1982).

In conclusion the available evidence suggests that EB virus is not aetiologically related to rheumatoid arthritis. However, the possibility remains that the defect of cellular immunity to EB virus is only one example of aberrant control of latent DNA viruses in RA patients and the appearance of certain "auto-antibodies" directed against nuclear constituents may simply represent an entirely appropiate response to the expression and release of

latent DNA virus encoded nucleoproteins. The recent demonstration that "autoantibodies" to the soluble cellular antigen "La" will immunoprecipitate ribonucleoprotein (rnp) particles containing EB virus encoded RNA's (EBER 1 and EBER 2) from EB virus infected cells, and similarly rnp particles containing adenovirus encoded RNA's (VAI and VA II) from adenovirus infected cells lends support to this hypothesis (Lerner et al 1982).

Role of host factors

Introduction

While there is no direct evidence for an exogenous triggering agent in RA or SLE there is considerable evidence that genetic factors confer susceptibility to both these diseases. Both RA and SLE are found with increased frequency in association with certain HLA-D and DR antigens and SLE is also associated with certain inherited complement deficiencies. Such associations are of course not unique to RA and SLE and a number of other examples are shown in Tables 9 & 10 (p145).

TABLE 9 HLA antigens and disease
(Adapted from Panayi 1982)

CLASS I	
DR2	Goodpastures syndrome, multiple sclerosis
DR2/3	SLE
D3/DR3	Coeliac disease, Graves, Addisons, chronic active hepatitis, M. gravis, primary Sjogrens syndrome
DR3	Juvenile onset diabetes mellitus
DR4	" " " " , RA, hydrallazine induced SLE
CLASS II	
B27	Seronegative spondarthritis & reactive arthritis
CW6	Psoriasis
A3	Haemochromatosis

TABLE 10 Complement deficiency and disease
(Adapted from Zvaifler 1981)

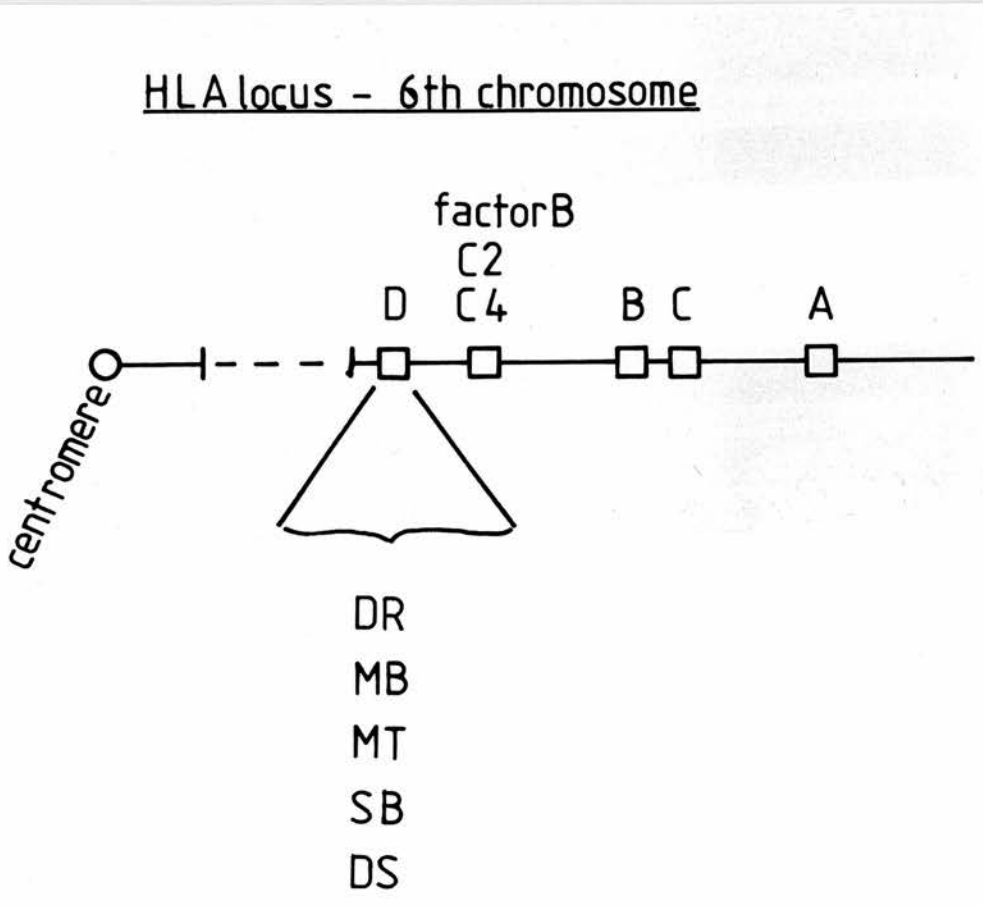
Deficiency	Association
Clq	combined immunodeficiency
Clr	glomerulonephritis, SLE, infection, arthritis
Cls	SLE
C4	SLE, normality
C2	normality, SLE, JCA, asthma, vasculitic purpura, nephritis, infection, RA
C3	serious infection
C5	Neisseria infection, SLE
C6	" " , normality
C7	" " , Raynauds/sclerodactyly
C8	" " , SLE
Cl INH	hereditary angiooedema, SLE
C3b INA	infection.

HLA system and disease

Since the HLA system has played a dominant role in recent studies of both RA and SLE, the structure and function of the HLA system will be briefly reviewed.

The major histocompatibility complex (MHC) is situated on the short arm of chromosome 6 and is divided into D, B, C and A loci (Figure 8).

Figure 8 The human leucocyte antigen (HLA) locus:



The complex also contains the genes coding for complement components C2, C4 and Factor B. The HLA genes exhibit considerable polymorphism but the complement genes exhibit one or two common alleles only. Furthermore the HLA antigens are very complex and each molecule carries multiple antigenic determinants, the sum of which identifies each gene product. The presence of multiple alloantigenic specificities suggests that there are several independently polymorphic regions on each molecule.

The class I genes (HLA-A, B, C) are analogous to the H2-K and D MHC genes of the mouse and their corresponding antigens are expressed on all nucleated cells in association with beta2 microglobulin (Howard 1978). The A, B and C antigens show considerable sequence homology suggesting that they have arisen by tandem reduplication of their corresponding genes. Furthermore A and B antigens also show sequence homology with IgG heavy chain suggesting that they are distantly related molecules. Similar homology is seen between beta2 microglobulin and immunoglobulin (Howard 1978). The principal function of the HLA-A, B and C antigens seems to be in cell-cell interactions and they play an essential role in cytotoxic T-cell lysis of viral infected cells (Snell 1978). They may also play a role in other cell-cell interactions (Meruela and Edidin 1980) and this may be the basis for the association of HLA-CW6 with Psoriasis.

The class II HLA-D (MLR defined) and HLA-DR (serologically defined) antigens are analogous to the I region antigens of the mouse and are expressed in a more restricted fashion principally on

macrophages, B-cells, some T-cells, spermatozoa and Langhans cells. The D/DR antigens are important in the initiation and induction of immunity and control macrophage-lymphocyte interactions and T-helper and suppressor cell functions. Following uptake and "processing"of antigen by macrophages, antigen is presented in association with Ia or HLA-D antigen to responding lymphocytes. Delayed type hypersensitivity (DTH) and T-helper cell responses are specific for small peptide sequences (3-4 amino acids) only (Benacerraf 1978); thus destruction of tertiary protein structure does not interfere with antigen recognition in a secondary DTH response while alteration of primary amino acid sequences abolishes recognition. This specificity may be conferred by the Ia or DR molecules which may act as receptor or binding sites for these foreign sequences. Mutual sharing of Ia or DR alloantigen is essential for antigen recognition; thus secondary proliferative responses of monocyte depleted T-lymphocytes to PPD are restored only when monocytes sharing identical HLA-D alleles with the T-cells are added whether or not the macrophages are from an unrelated donor (Sonderstrop et al 1978). Furthermore recognition of foreign antigen by responding lymphocytes is blocked by antisera against the D or Ia molecule. A further important point is that for certain antigens, immune response genes are expressed at the level of the macrophage and operate by specifying the antigenic determinant on a foreign molecule which are presented to the lymphocyte (Rosenthal et al 1977, 1979). Proliferative responses of T-helper cells to macrophages bearing antigen also require soluble

stimuli such as interleukin-1 (Fauci 1982). T-helper/B-cell interactions are also governed by histocompatibility restrictions and recognition of the antigen-Ia complex is essential for helper cell activity.

More recently a number of additional class II antigens have been identified and include the MB, MT, TE, SB and DS specificities. Whether these are products of distinct D-region genes or are "supertypic" specificities is not known (Rodnan & Schumacher 1983). Certain MB and MT types are very closely associated with certain DR antigens (Table 11, pl50) (Winchester & Nunez-Roldan 1982).

In addition to the HLA loci, the genes coding for C2, C4 and Factor B are also found in the MHC. Some hereditary complement deficiencies are in linkage disequilibrium with certain HLA genes; for example C2 deficiency with A10/B18/DW2/DR2 (Schur 1982).

TABLE 11 Relationship of novel class II alloantigen specificities to DR alloantigens.
(From Winchester & Nunez-Roldan 1982)

Novel alloantigen	Associated DR alloantigen								
	1	2	3	4	5	6	7	8	9
TE21	1	2							
MT1	1	2				6			
MB1	1	2				6		8	
TE23	1			4					
TE24			3				7		
MB2			3				7		
MT2			3		5	6			
TE22				4	5				
MB3				4	5	6			9
MT4				4	5			8	
MT3				4			7		9

Role of host factors in RA

Numerous studies have confirmed an association between seropositive RA and HLA-DR4 and some 60% of patients carry this antigen compared with about 30% of controls (Stastny 1980). Other D/DR associations include disease severity and side effects of penicillamine and sodium aurothiomalate therapy. Panayi et al (1978) demonstrated that RA patients with more severe disease, higher rheumatoid factor (RF) titres and nodules had an increased frequency of DRw3 while those with milder disease and lower RF titres were DRw2 positive. Furthermore there was an overall reduction of DRw2 in the RA group as a whole suggesting that DRw2 has a protective effect. Wooley et al (1980) found that a high proportion (19/24) of patients with proteinuria induced by penicillamine or sodium aurothiomalate were B8/DRw3 positive and that 13/13 with heavy proteinuria (>2gm/24 hours) were B8/DRw3 positive. Overall however the DR4 antigen is a relatively weak risk factor for RA with a relative risk of only 6-9X that of the general population.

Other genetic factors may also play a part in determining disease severity and an association between early onset severe erosive RA and heterozygosity for alphal antitrypsin deficiency has been noted (Cox & Huber 1980).

Role of host factors in SLE

The role of genetic factors in SLE has recently been well reviewed (Winchester & Nunez-Roldan 1982). Classical genetic studies of the occurrence of SLE in monozygotic and dizygotic twins clearly demonstrate the existence of a genetic predisposition. Analysis of twin pairs in which at least one twin had lupus revealed that in 69% of monozygotic twin pairs both members had clinical lupus while only 3% of dizygotic twin pairs were concordant for lupus. However since 31% of monozygotic twins did not have overt lupus, environmental factors are also necessary for disease expression. Calculations based on this data give an approximate estimate of four co-occurring disease susceptibility genes for expression of the disease. Studies of parent-child inheritance patterns suggest very strongly that the disease susceptibility is inherited as a dominant trait.

Further information on the nature of the gene products involved in susceptibility have come from population genetic studies of known polymorphic genes such as the HLA system. Pooled data from a number of North American studies show a highly significant increase in frequency and relative risk for DR2 and DR3 alleles with reduced relative risk for DR4 and DR5, and the association suggests that one of the susceptibility genes postulated in the classical family genetic studies is located within the MHC. A further important point is that DR2 and DR3 may be markers for one of the more recently identified nonDR class II alloantigens and it has been suggested that MT1 or MB1 may be the

relevant specificity. In support of this, a recent study has demonstrated a relative risk of 18.8 for the MT1 antigen. The curious combined association of DR2 and DR3 with SLE is not readily explained and some features of the disease such as antibody to dsDNA and Ro are associated with DR3 while others such as thrombocytopenia are associated with DR2. It is not known whether co-occurrence of DR2 and DR3 in the same individual is associated with increased disease susceptibility. The complexity of the MHC association is further illustrated by the fact that in European studies an association has been noted with DR3 but not DR2 while in the UK the association is reversed. This may be due to differences in population genetics and the relative genetic homogeneity found within European geographical regions as compared with North America where there may be greater genetic mixing.

Various inherited defects of the complement system have also been described in SLE (Table 10, p145) but the most common association noted is with C2 deficiency. There is an increased frequency of the C2 deficient heterozygous state in SLE and furthermore 37% of homozygous C2 deficient subjects develop SLE. It should be noted however that patients with SLE and homozygous C2 deficiency have a lower incidence of renal disease which probably reflects failure of classical pathway activation by immune complexes (Schur 1982).

The mechanism of the association between complement deficiency and SLE is not entirely clear. Early components such as C2 play a role in viral neutralisation and deficiency could predispose to

viral infection. It is also possible that failure of solubilisation of immune complexes by complement (Miller & Nussenzweig 1975) could predispose to immune complex disease. However the association of SLE with heterozygous C2 deficiency in which C2 levels are generally adequate suggests that C2 deficiency is merely a genetic marker rather than a biological cause of SLE. This hypothesis is supported by the existence of linkage disequilibrium between C2 deficiency and HLA-A10,B18,DW2,DR2 haplotype.

An illustration of the way in which several genes may interact with an environmental agent to produce SLE is hydrallazine induced lupus. Studies by Batchelor et al (1980) have clearly shown that slow acetylator status co-occurring with HLA-DR4 in a female is associated with a very high incidence of hydrallazine induced SLE. An intriguing aspect of this work is the finding that DR4 is a risk factor, whereas in spontaneous SLE DR4 has a protective effect with a relative risk of -2.2.

Immune complex formation in RA and SLE

Introduction

Although the initiating causes of RA and SLE are unknown, it is clear that a major pathogenetic mechanism in both diseases is the production of immune complexes (IC) which interact with inflammatory cascades, neutrophil polymorphs, and other inflammatory effector cells to cause tissue damage. A complete discussion of the nature and biological properties of the immune complexes found in RA and SLE is beyond the scope of this thesis, but there are some basic concepts which should be reviewed.

General considerations

Formation of ICs by reaction of antibody with antigen is reversible and obeys the law of mass action. The affinity of the antibody is a major determinant of the equilibrium constant, and low affinity antibody shifts the equilibrium to the left i.e. towards dissociation of the IC.

$$Ab + Ag = (Ab\text{–}Ag)$$

The nature of the antigen, including its size, charge and the number and accessibility of antigenic determinants also determine the stability and biological properties of the IC. Studies of inbred strains of mice with high and low affinity antibody

responses have shown that "low" responders are much more susceptible to chronic IC disease and glomerulonephritis (Steward & Devey 1981). It has been proposed that this type of genetically determined, antigen nonspecific chronic disease should be viewed as part of the spectrum of immunodeficiency (Soothill & Steward 1971). The genetic basis for the affinity of antibody responses is likely to be complex, and may involve macrophages (Passwell et al 1974; Morgan & Soothill 1975), T-helper cells (Soothill & Steward 1971) or B-cells (Steward et al 1974). Furthermore, macrophage clearance defects (Passwell et al 1974; Morgan & Soothill 1975) while predisposing to defective antigen handling and low affinity antibody responses, may also contribute to defective clearance of the ICs so produced.

Immune complexes in RA

A major immunological feature of RA is the production of rheumatoid factors (RF) which are antiglobulins directed against the Fc portion of IgG. The target for RF on the Fc portion is probably the isotypic Ga autoantigenic determinant (Johnson &Faulk 1976). RFs are predominantly of IgG and IgM classes although all classes have been described. Although the stimulus to RF production is unknown, its inevitable consequence is the formation of ICs produced by the reaction of RF with native IgG. In the plasma, the ambient concentration of native IgG is relatively high and small di- and trivalent complexes tend to form between IgG class RFs and native IgG. These are low in inflammatory potential and do not fix complement, do not trigger inflammatory cells and bind poorly to IgM RF. With increasing titres of IgG RF, the complexes tend to increase in size, fix complement, are more readily crosslinked by IgM RF and trigger inflammatory cells (Carson et al 1979; Weisman & Zvaifler 1975). Under these circumstances, intravascular inflammation may be triggered by deposition of ICs in small arterioles (Conn et al 1972) and is manifested clinically as vasculitis affecting a variety of organs including skin, nerves, eyes and lung, and pathologically by inflammatory necrotising lesions of the blood vessel walls. The presence of circulating inflammatory ICs is also marked by falling levels of haemolytic complement, consumption of C3 and rising levels of complement degradation products (Mongan et al 1969; Weinstein et al 1972).

In general however, inflammatory events in RA occur

predominantly in the extravascular space such as the synovial and serosal cavities, and are triggered by the local synthesis of RFs (Zvaifler 1973). The factors which lead to the accumulation of lymphoid cells and RF synthesis are not known, but once established, a self perpetuating sequence of IC formation and inflammation occurs. RFs synthesised in extravascular sites have no native IgG to react with and therefore form large self associating polymers which readily trigger inflammation. Rheumatoid synovial fluid is therefore characterised by intense complement consumption which attracts neutrophil polymorphs and mononuclear cells while the synovial lining becomes hypertrophied with increasing numbers of infiltrating lymphocytes and macrophages (synovial A cells) (Zvaifler 1973). The interactions of these chronic inflammatory cells with humoral factors and other connective tissue cells to cause connective tissue degradation has already been discussed (p115).

It is likely that in relation to IC formation and clearance in RA, Kupffer cells and splenic macrophages may be most relevant to protection against intravascular inflammatory events, while in joints and extravascular spaces, the resident synovial A cells and blood derived mononuclear phagocytes will be more important.

Immune complexes in SLE

In contrast to RA, a large proportion of the inflammatory organ damage seen in SLE is caused by intravascular deposition of ICs, formed either locally or in the circulation, and the basic pathological event is therefore vasculitis. This is reflected both in the nature of the tissue damage as well as in the diversity of organ involvement. Thus while in RA, joint erosions and destruction by recurrent "Arthus-like" reactions are common, in SLE, although synovitis is frequent, erosions are rare. Similarly, while in SLE any organ can be involved, in RA certain organs such as the brain and kidney are only rarely involved by the inflammatory process.

The diversity of autoantibodies found in SLE creates the potential for IC formation with a wide range of antigenic determinants in the membranes, cytoplasm and nuclei of cells, as well as soluble antigens such as globulins, and at least three different mechanisms of IC production can be identified.

For example antibody against "solid phase" determinants on erythrocytes and platelets contribute to haemolytic anaemia and thrombocytopenia and it has been suggested that autoantibodies directed against neuronal tissue may mediate some features of CNS disease in lupus (Zvaifler & Bluestein 1982). It is likely that the ability of anti-neuronal antibodies to fix complement and activate the membrane attack complex (MAC) is a critical requirement for pathogenicity of these antibodies (Koffler et al 1982). A second mechanism of immune complex formation is for circulating antibody to react with antigen which has been released from damaged tissue

and has become deposited in a site of predilection elsewhere in the body. Evidence for this mechanism comes from animal models of glomerulonephritis (Mannik 1982). The third mechanism of immune complex formation is via reaction of antibody with antigen in the fluid phase, and transport via the circulation to sites of tissue deposition. At least one antigen involved in this type of mechanism is DNA (Agnello & Mitamura 1982), and IC deposition has been demonstrated in a wide variety of tissues including kidney, skin, lung, spleen, bladder and placenta (Brentjens & Andres 1982) as well as choroid plexus (Zvaifler & Bluestein 1982). It should be emphasised however that demonstration of deposits of immunoglobulin and complement do not always correlate with tissue damage and it has been suggested that the ability of ICs to activate the MAC may be critical in this regard (Koffler et al 1982).

In conclusion, although many of the pathological features of SLE can be attributed to tissue deposition of ICs, there are a variety of potential pathogenetic mechanisms which do not necessarily involve circulating preformed ICs. Thus the clearance function of the splenic and hepatic components of the mononuclear phagocyte system may not be relevant to all manifestations of the disease process. Where local formation of ICs is occurring the function and local accumulation of blood derived monocytes and macrophages may be most important in removing ICs.

Mononuclear phagocyte system (MPS) immune clearance function

Introduction

Since RA and SLE are diseases in which immune complexes play a central role in the pathogenesis of inflammatory tissue damage, the function of the MPS in sequestering potentially harmful immune complexes (IC) is of great importance and has been studied in both these diseases as well as in animal models of IC disease. Studies in animal models have focussed on the factors which modulate MPS function and the characteristics which lead to their clearance or deposition in the tissues. In man a variety of model ICs have been used to examine the effect of IC disease on the MPS and to determine whether MPS dysfunction plays a significant role in the persistence of ICs. Other studies have been performed on monocytes and macrophages in vitro with the aim of determining whether phagocytic defects are present in cells taken from patients with IC disease or, alternatively, whether serum from these patients induces defects in phagocytic cells from normal subjects.

In vivo studies of (MPS) function in animals

Animal models have demonstrated that handling and clearance of circulating immune complexes (IC) is a function both of MPS function and the immunochemical characteristics of the ICs. The concentration of circulating IC at a given moment is a function of the rate of formation and removal. Formation of ICs is dependent both on the availability of antigen, the rate of antibody synthesis and the overall affinity of the antibody "population" (Steward & Devey 1981). Removal of ICs by the MPS is dependent on the IC lattice size, the type of antigen, the characteristics of the antibody and the status of the MPS (Mannik 1982).

The role of lattice size in MPS immune clearance has been studied by infusing preformed ICs of known size into experimental animals (Haakenstad and Mannik 1976; Mannik et al 1971; Finbloom and Plotz 1979a, 1979b). "Large" lattices are defined as complexes containing more than two antibody molecules (i.e.>Ab2Ag2) and "small" lattices as complexes containing Ab2Ag2 or less. After infusion, large complexes are cleared rapidly in an exponential fashion while small complexes are cleared much more slowly. Saturation of clearance mechanisms by increasing the dose of ICs can be achieved only with the use of large lattice complexes and no plateau in clearance is seen with increasing amounts of small ICs (Haakenstadt and Mannik 1974, Mannik et al 1971). Furthermore whereas hepatic uptake of small complexes causes no inhibition of MPS clearance of aggregated IgG, large lattice ICs readily cause

"blockade" (Jimenez et al 1983). Clearance of such complexes is entirely mediated by the Kuppfer cells of the liver which are ideally suited for this purpose. The Kuppfer cell membrane and its receptors are exposed directly to the blood stream and unlike the splenic macrophage have no covering of endothelium. The spleen on the other hand clears particulate material such as erythrocytes which must enter the extravascular space before making contact with specific phagocytic cells.

The antigen contained in the immune complex also plays a role in clearance - uncomplexed ssDNA for example is cleared more rapidly than large lattice ICs in mice (Emlen and Mannik 1978). The structure and type of antibody also plays a key role. For example reduced and alkylated ICs with damaged Fc portions and mouse IgA complexes which are not bound by macrophage Fc receptors have slow rates of clearance and become deposited in the glomerular mesangium causing nephritis.

A number of factors alter the innate capacity of the MPS to clear ICs and include prior blockade by ICs (Haakenstad and Mannik 1974), drugs (Haakenstad et al 1975), intrinsic strain specific factors (Ford 1975) and treatment with agents which activate the MPS (Raij et al 1981, Barcelli 1981).

As discussed already, blockade of the MPS can be achieved using large lattice ICs but not small lattice ICs (Jimenez et al 1983), an observation which is of some importance vis a vis the significance of small soluble ICs circulating in human disease. The effect of steroids on monocyte phagocytosis is controversial and

has been discussed already (vide supra) but whatever the mechanism steroids do seem to impair in vivo hepatic clearance of ICs (Haakenstad et al 1975) and thus secondarily increase the incidence but not the severity of glomerulonephritis in animal models. The observation that steroids ameliorate splenic clearance defects in man must be contrasted with these observations and suggests that blood flow and vascular permeability may play a role in these differential effects on organ IC clearance. Animal studies (Ford 1975) have also demonstrated strain differences in MPS function which can be related to a disposition to IC mediated glomerulonephritis. However it should be noted that in animal models of IC mediated glomerulonephritis, there is an association with defective clearance in some but not all studies (Finbloom and Plotz 1979, Pappas et al 1981, Hoffsten et al 1979) and other mechanisms such as antigen deposition followed by in situ IC formation may also play a role (Ford and Kosatka 1979).

Several studies have demonstrated that MPS clearance function can be significantly enhanced by MPS activating agents. Atkinson and Frank (1974) demonstrated that following BCG immunisation the pattern of complement mediated clearance of ICs by guinea pig Kuppfer cells altered. Prior to immunisation complement coated erythrocytes were rapidly cleared by the Kuppfer cells but later released back into the circulation, whereas after BCG the cells were cleared completely. Two other studies (Raij et al 1981, Barcelli et al 1981) have shown that prior injection of zymosan or C. parvum into animals ameliorates or prevents the development of

glomerulonephritis due to passively infused ICs.

Clinical studies of MPS function in vivo in RA, SLE and other autoimmune diseases

Early studies of human "RES" function using lipid emulsions showed enhanced clearance in patients with SLE, RA, rheumatic fever and other inflammatory diseases (Salky 1965).

Others have used heat damaged autologous erythrocytes to study splenic "nonimmune" phagocytic function in vivo. Williams et al (1979) demonstrated reduced clearance in 11/13 patients with active RA and a correlation between reduced clearance and immune complex levels (Clq binding activity). 13 patients with inactive RA, some of whom were on penicillamine or gold therapy, had normal splenic function. Henderson et al (1981) and Gordon et al (1981) using the same technique demonstrated minor reductions in erythrocyte clearance in 3/14 and 8/20 patients respectively. In neither study was a correlation found with disease activity or Clq binding activity. A study of 10 patients with SLE (Elkon et al 1980) identified only one patient with delayed erythrocyte clearance while another patient with normal clearance had very high circulating IC levels.

It is likely that this technique is measuring the capacity of the spleen to filter out deformed spherocytic erythrocytes which are sequestered primarily because of lack of deformability (Mohandas et al 1979). Studies in vitro on heat damaged erythrocytes show alterations in size (usually increases) and no evidence to suggest that they become opsonised with immunoglobulin

or complement. It is not known however whether other opsonins such as fibronectin play a role in the in vivo clearance of these particles and the clinical significance of these studies is uncertain.

In an attempt to test immune Fc receptor mediated clearance mechanisms, chromium labelled autologous erythrocytes opsonised in vitro with human anti-Rh(D) IgG antibody have been used as artificial ICs. Anti-Rh(D) do not fix complement (Rosse 1968) and their rate of clearance is proportional to the degree of opsonisation (Elkon et al 1982). A number of studies on patients with SLE have demonstrated that a high proportion of patients have defective MPS clearance of these particles and this has been attributed to abnormal Fc receptor function. Frank et al (1979) reported that 13/15 SLE patients had reduced Fc mediated clearance and 6 of these patients had normal or even increased clearance of nonimmune particles (radioiodine labelled aggregated albumin). Reduced Fc mediated clearance correlated with increased disease activity and weakly with Clq binding activity (Clqba). A subsequent study compared subgroups of SLE patients with and without renal disease (Parris et al 1982). Again SLE patients had slower rates of clearance than normal controls but in addition, patients with renal disease had slower rates than those without. However, the differences were not great and no control group of patients with other nonimmune causes of renal failure was included. No correlation was found between clearance and anti-DNA antibodies, or tests for ICs (Clq ba, Raji cell and Staph A binding assays). A

study of 19 patients with Sjogrens Syndrome demonstrated reduced clearance in 12/19 patients and a correlation between the defect and disease activity (Hamburger et al 1979). In general defects of Fc clearance were seen in those with extra-salivary gland disease but again no correlation was found with circulating ICs or serum complement levels.

Two recent studies have reported the results of longitudinal tests of MPS Fc receptor mediated clearance in patients with SLE. Hamburger et al (1982) studied 13 patients, 11 of whom had diminished Fc mediated clearance the severity of which correlated with disease activity and tests for immune complexes. Paired studies on these patients demonstrated that clinical improvement was associated with improvement in clearance rates and, in one patient whose disease flared, clearance rates became slower. Tests for ICs also changed concordantly. Kimberley et al (1983) similarly reported concordance between improvement in disease and improved clearance but found no concordance with tests for ICs including CH50, Staph. A binding, Clqba and Raji cell assays. Although it appears that the magnitude of the defect of Fc clearance in SLE alters with time and disease activity there is no agreement as to whether this correlates with levels of ICs and most studies have found no or only weak correlations.

Reduced Fc mediated clearance has also been reported in patients with nephritis and vasculitis, which has improved following plasma exchange (Lockwood et al 1979). Very little data is available on patients with RA, one report Kimberly and Ralph

(1983) suggesting minor reductions in Fc mediated clearance in a few patients with active disease.

The basis for the defect of putative Fc mediated MPS clearance demonstrated in these studies is necessarily speculative. Clearance is a function of a number of factors including organ blood flow and size, as well as the state of activation of the MPS. Clearance may also be modified not only by "saturation" of clearance mechanisms (Haakenstad and Mannik 1974) but also by depletion of soluble circulating fibronectin, and pharmacological factors. In acute severe illness such as trauma and septicaemic shock, profound abnormalities of blood flow and "RES" clearance have been noted which are associated with depletion of circulating fibronectin, an opsonic glycoprotein. Transfusion of fibronectin rich cryoprecipitate results in dramatic improvement in blood flow and clearance (Saba 1980). The difficulty in demonstrating a correlation between clearance and ICs is perhaps not surprising as animal studies have shown that large complexes which are rapidly cleared can induce MPS "blockade" whereas small circulating ICs do not (Mannik 1982). Thus circulating ICs may not be representative of ICs being actively removed by the spleen. In patients with subacute bacterial endocarditis with clinical and serological evidence of circulating ICs, Fc receptor mediated clearance is enhanced rather than depressed, and again emphasises the difficulty in trying to associate the presence of circulating ICs with MPS clearance defects. The enhancement almost certainly reflects "activation" of the MPS and is comparable to observations made in

animals (Atkinson and Frank 1974, Raij et al 1981, Barcelli 1981).

A further question in the interpretation of these studies is whether clearance of IgG sensitised erythrocytes represents an immune clearance mechanism relevant to IC disease such as RA or SLE. Soluble ICs bearing both complement and Fc-portions or other ligands are cleared in the liver rather than the spleen. This has been demonstrated in the various animal models already discussed as well as in human studies (Atkinson & Frank 1974). Furthermore the Kuppfer cell is ideally suited for this purpose as it lies in direct contact with plasma as it circulates through the liver. The use of a particle coated both with complement and a second, phagocytic ligand (carbohydrate or Fc-portion) which would be phagocytosed by Kuppfer cells would be a more physiological probe of MPS clearance function.

In addition to these studies which have essentially considered defective splenic clearance as a secondary manifestation of IC disease it has been suggested by analogy with animal models (Ford 1975), that there may be intrinsic defects of MPS receptor function which predispose to IC disease. Lawley et al (1981) demonstrated defective splenic clearance in a group of normal individuals with the B8/DRw3tissue type and suggested that this might represent an inherited predisposition to abnormal MPS function. None of these individuals had any evidence of circulating ICs or serological evidence of immunological disease. However it has since been reported that blood monocytes from individuals with the B8/DRw3 tissue type have normal numbers of Fc receptors with normal

affinity making it extremely unlikely that abnormal Fc clearance is due to an intrinsic defect of Fc receptor expression. These observations must cast further doubt on the validity of the technique as well as the method of selecting "normal controls".

C3b receptor MPS clearance

Studies have also been performed to try and delineate the role of complement receptors in mediating in vivo clearance of circulating ICs in man. Autologous chromium labelled erythrocytes coated with IgM antibody and C3b are rapidly cleared by the Kuppfer cells of the liver (Atkinson and Frank 1974). After the initial clearance phase, a proportion of the labelled cells are released back into the circulation where they have a normal survival time as C3d coated Coombs positive erythrocytes. Kinetic studies demonstrate that rates of clearance are proportional to the degree of sensitisation (Meryhew and Runquist 1981). In animal models a similar pattern of clearance and release has been observed but which can be enhanced by prior MPS activation (vide supra)(Atkinson and Frank 1974).

Reports of clinical studies are limited. One report demonstrates reduced C3b mediated clearance in primary biliary cirrhosis and no abnormality in controls with other forms of liver disease (Jaffe et al 1978). In addition these patients did not have a defect of nonimmune clearance of aggregated albumin and 4/6 had increased rates of Fc mediated splenic clearance. No correlation between delayed C3b clearance and circulating ICs was noted.

Other approaches to the study of C3b receptors have been used and several groups have reported evidence of defective C3b receptor (CR1) expression in erythrocytes and renal glomerular cells. Miyakawa et al (1982) have reported that over 60% of a group of 56 patients with SLE had very low densities of CR1 expression on their erythrocytes as detected by immune adherence and antiCR1 antibody and that the abnormality did not improve with amelioration of the disease. Moreover a similar abnormality was found in a high proportion of a small group of relatives of these patients but only a small proportion of the normal controls. On this basis they suggest that the defect may be intrinsic or inherited. However they did not satisfactorily exclude the possibility that the abnormality might be acquired as a result of exposure to complement bearing ICs. Iida et al (1982) similarly found reduced CR1 expression using anti-CR1 antibody on erythrocytes from patients with SLE and RA and that the defect in SLE patients correlated with C4 haemolytic titres and C1qba. Although the data suggests that the abnormality is secondary, the data does not preclude a genetic component.

A larger study of erythrocyte CR1 expression in 113 normal subjects, 38 patients with SLE, four of their spouses and 47 relatives has also been reported (Wilson et al 1982). Anti-CR1 antibody and C3b dimers were used to quantify receptor numbers. The frequency of distribution of CR1 expression in normals suggested a trimodal pattern which was interpreted as representing three phenotypes - 34% had high (HH), 54% intermediate (HL) and 12% low (LL) phenotypes respectively. The observed frequencies correspond

well to the predicted frequencies of a two codominant allele model of inheritance. The mean number of CR1 on erythrocytes from both SLE patients and their relatives was significantly lower than controls, but their spouses resembled the normal subjects. Furthermore they excluded the possibility of receptor occupancy by ICs as well as differential effects due to maturity of erythrocytes.

The functional significance of CR1 on erythrocytes vis a vis the MPS is two fold. Firstly the erythrocyte bears approximately 85% of cellular CR1 in the peripheral blood and may therefore play a significant role in transporting ICs to the MPS. Nelson (1956) observed that virtually 100% of pneumococci injected with antibody into monkeys became associated with erythrocytes and were cleared completely from the plasma. Cinematographic studies demonstrated polymorphs removing bacteria from the surface of erythrocytes leaving the erythrocyte intact (Robineaux and Pinet 1960) suporting the concept that erythrocytes may play a significant role in immune clearance.

The second implication of an inherited defect of CR1 expression on erythrocytes is that other marrow derived cells including mononuclear phagocytes may be similarly affected, again with important consequences for the clearance of opsonised ICs. In support of this hypothesis, immunohistochemical studies of the human glomerulus using anti-CR1 antibody have shown that CR1 is absent from diffuse proliferative nephritic SLE kidneys, but is present in kidneys with several other forms of glomerulonephritis

including membranous SLE nephritis (Kazatchkine 1982). Furthermore data to be presented in this thesis also suggests that blood monocytes from patients with SLE have defective C3 receptor expression.

Clinical studies of MPS phagocytic function in vitro in RA and SLE

Introduction

Early studies used Roebuck skin window techniques to study the phagocytic function of exudate inflammatory polymorphs and macrophages in RA. Jessop et al (1973) found that the phagocytic activity of macrophages and neutrophil polymorphs was increased in RA and suppressed during treatment with gold salts or steroids. Parallel experiments on rats confirmed the effects of gold and prednisolone and showed that these drugs had a gross inhibitory effect on both humoral and cellular components of inflammation (Vernon-Roberts et al 1973). However while these studies provide a useful overall view of the inflammatory response, individual components such as adherence, chemotaxis, spreading and phagocytosis and the contribution of humoral factors cannot be separated. In general most subsequent studies have employed blood monocytes in various degrees of purity to study phagocytic function.

Fc receptor expression in SLE and RA

There have been relatively few studies of phagocytosis by monocytes from SLE patients and most have examined the role of serum opsonisation factors (Svensson 1975, 1980a, 1980b, 1980c; Svensson and Hedburg 1973; Temple and Loewi 1977). Kavai et al (1979, 1980, 1983) have demonstrated enhanced Fc receptor (FcR) expression in monocytes from SLE patients and also shown that this

returns to normal after incubation in vitro for four hours (Kavai 1980). The enhancement of FcR was correlated with several facets of monocyte function and activation, including increased β-glucuronidase content and a rise in serum lysozyme levels.

Studies of RA patients (Molleraimussen et al (1982)) using radio-iodine labelled monomeric IgG demonstrated a significant increase in numbers of FcR/monocyte in 14 untreated patients compared with 15 normal controls. Katayama et al (1981) similarly found increased numbers of FcR/monocyte and evidence of increased catabolism of aggregated IgG in RA monocytes. The increase in FcR correlated with rheumatoid factor latex titres. Hoch and Shur (1981) found increased EA rosetting and increased Fc recptor dependent phagocytosis but no increase in uptake of aggregated IgG. FcR rosetting and phagocytosis by monocytes from patients on steroids were no different from the controls. Conversely however they found that incubation of normal monocytes with RA sera caused reduced rosetting and phagocytosis. Other functional studies demonstrating increased ADCC by RA monocytes also suggest increased Fc receptor expression (Waytz and Douglas (1979)).

Enhanced FcR expression on monocytes has been noted in a number of other chronic inflammatory diseases including sarcoidosis, Crohns disease and tuberculosis, and has been attributed to monocyte activation (Zuckerman and Douglas (1979)). However the functional significance of this increase is uncertain and it is not clear whether it results in increased phagocytic activity or whether the FcR mediate other functions such as

superoxide generation or cytotoxic effects. The available evidence suggests that the latter is the correct interpretation but more information with regard to the type of Fc receptor involved and its function is clearly required.

A comparison of in vivo MPS function with in vitro monocyte function was recently reported (Salmon and Kimberly 1983). Small but significant reductions in monocyte Fc mediated phagocytosis but increased Fc rosetting with EAIgG were found. Measurement of FcR numbers using radio-labelled aggregated IgG showed a small but statistically insignificant increase in FcR expression and no change in affinity. There was no correlation between these in vitro findings and in vivo MPS clearance of IgG sensitised erthrocytes.

Complement receptor expression in SLE and RA

In two studies of SLE patients defects of phagocytosis of serum opsonised bakers yeast were demonstrated and attributed to an abnormality of C3b receptor expression (Kavai et al 1979, 1980). Landry (1977) using an oil emulsion of E. coli lipopolysaccharide as the test particle and normal serum as the opsonising agent found reduction of initial rates of phagocytosis by both neutrophil polymorphs and monocytes from patients with SLE. Although it is not specified which opsonins were present on the emulsion, it seems likely that C3b generated by alternative pathway activation was a major opsonic component and that C3b receptors were responsible for the phagocytic defect.

In a study of RA patients Hoch and Shur (1981) found no change

in monocyte complement (C3b) receptor expression using EAC rosettes.

In conclusion, there is evidence from in vivo studies of SLE and to a lesser extent RA patients, to suggest impaired Fc-receptor mediated clearance by splenic macrophages. However in vitro studies of their precursors, blood monocytes, which are presumably exposed to the same humoral environment, show definite enhancement of Fc receptor expression. This apparent contradiction may reflect differences in methodology. In rosetting studies no indication of the functional phagocytic capacity of Fc receptors is obtained, and increased numbers of Fc receptors cannot be equated with increased phagocytic capacity. In vivo studies are complicated by a number of factors and the end result i.e. disappearance of erythrocytes from the circulation, may not directly reflect macrophage receptor function. Perhaps the clearest indication that monocyte immune phagocytic function is abnormal in SLE is given by studies using opsonised oil emulsion or yeast which suggest that complement receptor expression may be defective, and this is supported indirectly by the recent demonstration of abnormal CR1 expression on erythrocytes from SLE patients.

The question of whether there are primary or secondary defects of MPS phagocytic function which might contribute to persistence of immune complexes in SLE and RA is by no means resolved and it was in this context that the studies of monocyte phagocytic function reported in this thesis were undertaken.

CHAPTER 4

AIMS OF THE STUDY

AIMS OF THE STUDY

The principle aim of these studies was to determine whether defects of immune phagocytosis by mononuclear phagocytes could be identified in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) which might exacerbate or predispose to immune complex disease. The potential significance of abnormalities of immune phagocytosis, if present, is discussed in the preceding chapter. Since blood monocytes are the precursors of tissue macrophages it seemed appropriate to examine the function of these cells as a first step towards achieving this aim. Furthermore if intrinsic defects of the mononuclear phagocyte are present in either of these diseases they should be apparent in precursor cells as well as mature tissue macrophages and would perhaps be less likely to exhibit abnormalities secondary to local disease processes.

The experiments performed fall into the following categories.

1) The establishment of methods for measuring the kinetics of phagocytosis by blood monocytes which would be suitable for use in a clinical setting.

2) The application of these methods to the study of blood monocytes from patients with RA and SLE .

3) Correlation of phagocytic data with serological parameters including serum complement levels and tests for immune complexes.

4) Further studies aimed at elucidating the nature of any phagocytic abnormalities found in monocytes from patients.

CHAPTER 5

METHODS

SECTION I: BUFFERS , STAINS AND IDENTIFICATION OF MONOCYTES

1.0 BUFFERS

SORENSENS PHOSPHATE BUFFER.

M/15 disodium hydrogen phosphate was prepared by dissolving 9.465gm in 1 litre of distilled water, and M/15 potassium dihydrogen phosphate by dissolving 9.08gm in 1 litre of distilled water. Mixing of these two solutions in various proportions gave buffer of the required pH.

HANKS BALANCED SALT SOLUTION

Hanks balanced salt solution (HBSS) was adjusted to pH 7.4 with 1.4% sodium bicarbonate and preservative free heparin (5 units/ml) added.

HEPES.

1M Hepes buffer was prepared by dissolving 23.83gms of Hepes buffer in 90mls of distilled water and the pH adjusted to 7.6 with 5N NaOH. The final volume was adjusted to 100mls with distilled water.

EAGLES MINIMUM ESSENTIAL MEDIUM.

Eagles minimum essential medium (MEM) was prepared by dilution of 10 times concentrated Eagles MEM (Flow Laboratories), with the addition of 1ml of 0.2M glutamine, 0.1ml of penicillin and streptomycin (200,000 units/ml), 3ml of 1M HEPES, and adjustment of the final volume to 1 litre with sterile distilled water.

RPMI 1640.

RPMI 1640 + 2gm/l $NaHCO_3$ (Flow Laboratories) (X1 conc) was supplemented with 1ml of 0.2M glutamine, 0.1 ml Penicillin and Streptomycin (200,000 units/ml).

FETAL CALF SERUM

A single batch of fetal calf serum (FCS) (Flow Laboratories) was used throughout.

EDTA

To prepare a 4.5% solution of EDTA, 4.5gms of ethylene diamine tetra-acetic acid (disodium salt) were dissolved in 100mls of phosphate buffer and adjusted to pH 7.4 with 1N NaOH.

2.0 STAINS

WHITE CELL COUNTING FLUID

White cell counting fluid was prepared by dissolving 0.1gm of gentian violet in 1 litre of 2% acetic acid.

TRYPAN BLUE

0.9mls of a solution of 0.4% trypan blue in PBS was mixed with 0.1mls of cell suspension.

JENNER-GIEMSA STAIN

Slides were fixed for 5 minutes in methanol, stained for 5 minutes in Jenners stain diluted 1:1 with buffer (pH 6.8), for 10 minutes in Giemsa diluted 1:10 with buffer (pH 6.8) and finally washed with buffer (pH 6.8).

NON SPECIFIC ESTERASE STAIN

Monocytes were stained for non specific esterase according to the method of Yam et al (1971).

MATERIALS:

1) Stock fixative was prepared by dissolving 200mg of Na_2HPO_4 and 1gm of KH_2PO_4 in 450mls of acetone, 250mls of formalin and 300mls of distilled water. The fixative was stored at 4C.

2) Stock pararosanilin solution was prepared by dissolving 2gm of pararosanilin in 50mls of 2N HCL with warming.The solution was cooled and filtered.

3) Stock sodium nitrite solution was prepared by dissolving 8gm of sodium nitrite in 200mls of distilled water.

4) Stock M/15 phosphate buffer pH 7.4 (Sorensens).

5) A fresh solution of 100mg of alpha napthyl acetate in 5mls of 2-methoxyethanol was prepared daily as required.

6) A 2% aqueous solution of methyl green extracted with chloroform was used as the counterstain.

METHOD:

Fresh cytocentrifuge slides were prepared using 200ul of mononuclear cells at a concentration of 10^5 /ml. The slides were air dried, fixed for 30 seconds at 4°C, washed three times with distilled water and dried. 36mls of phosphate buffer were mixed with 2mls of the alpha napthyl acetate solution in a Coplin jar. 2mls of stock pararosanilin solution were mixed with 2mls of stock sodium nitrite and after 1 minute 2.4mls of this were added to the Coplin jar. The mixture was adjusted to pH 6.1 and filtered before use. The slides were then stained for 30 minutes, washed thoroughly with distilled water and counterstained with 2% aqueous methyl green for 20 minutes.

AS-D CHLOROACETATE ESTERASE STAIN.

MATERIALS:

1) AS-D chloroacetate (Sigma).

2) Fast blue BB salt (Sigma).

3) N-N dimethyl formamide (Sigma).

METHOD:

Fresh cytocentrifuge slides were prepared and fixed as for NSE staining. Where dual NSE/chloroacetate staining was required, the chloroacetate stain was performed after NSE staining but before counterstaining with methyl green.

10mg of AS-D chloroacetate are dissolved in 5mls of dimethyl formamide in a glass tube. 2mls of this solution are mixed with 38mls of M/15 phosphate buffer and 40mg of fast blue BB salt in a Coplin jar. The cytocentrifuge slides are stained for 20 minutes, rinsed with distilled water and counterstained with 2% methyl green for 20 minutes.

3.0 COULTER SIZING STUDIES OF MONONUCLEAR CELLS

A ZBI Coulter counter was used to perform studies of the size distribution profile of blood mononuclear cells.

CALIBRATION OF COULTER COUNTER

A ZBI Coulter Counter fitted with a 70 micron aperture was calibrated according to the makers instructions using the "half count method" on standard latex particles of known volume. Latex particles (Coulter) of mean diameter 13.8 and 4.84 microns were used and the calibration constant obtained from the formula:

$$(K/d) = (A \times I \times t) ;$$

where K = calibration constant, d = diameter of test particle, A = amplification, I = current, t = threshold setting.

Having determined the calibration constant using particles of known volume, the volume and number of particles of unknown size in suspension can then be obtained.

4.0 IMMUNOFLUORESCENCE STUDIES

IMMUNOFLUORESCENCE STUDIES ON MONONUCLEAR CELLS

Cytocentrifuge slides of mononuclear cells were dried and fixed in absolute alcohol for 15 minutes. After thorough drying the cells were stained with a few drops of diluted (1 in 20) FITC labelled goat anti-human gamma globulin or anti-human complement (βlc/a) (Nordic Immunodiagnostics) for 20 minutes at room temperature in a humidified chamber. The slides were then washed three times with PBS and mounted in 75% glycerol in PBS under a coverslip. The slides were examined under the fluorescence microscope using PLOEM illumination (Leitz Ortholux) for the presence of intracytoplasmic inclusions of gamma globulin or complement.

IMMUNOFLUORESCENCE STUDIES ON PREOPSONISED YEAST

Cytocentrifuge slides of preopsonised yeast were fixed in absolute ethanol for 15 minutes. After thorough drying the yeast were stained with a few drops of diluted (1 in 20) FITC labelled goat anti-human IgG, IgM, IgA or complement (βlc/a) (Nordic Immunodiagnostics). The slides were then washed three times with PBS and mounted in 75% glycerol in PBS under a coverslip. The slides were examined under the fluorescence microscope.

Appropriate positive and negative controls were used throughout.

SECTION II PURIFICATION OF CELLS

1.0 SEPARATION OF MONONUCLEAR CELLS ON A DENSITY GRADIENT

Mononuclear cells (monocytes and lymphocytes) were separated according to the method of Boyum (1968). 10mls of venous blood were anticoagulated with 2.0mls of 4.5% EDTA and placed in a 40ml siliconised glass centrifuge tube (M.S.E.). 14mls of Ficoll-Hypaque (Lymphoprep, Nyegaard, Oslo) was layered under the blood using a plastic quill and 20ml disposable plastic syringe. The blood was then centrifuged at 800g for 30 minutes at 20°C. The upper platelet rich plasma was discarded and the mononuclear cell layer collected along with approximately half the underlying Ficoll-Hypaque. The mononuclear cells were diluted with 30mls of HBSS+0.1% gelatin and centrifuged at 400g for 20 minutes at 20°C. The supernatant was discarded, the pellet resuspended in 5mls of HBSS+0.1% gelatin and the cells centrifuged at 100g for 10 minutes at 20°C. The pellet was finally resuspended in the required volume.

2.0 MONOCYTE PURIFICATION USING MICROEXUDATE COATED FLASKS.

PREPARATION OF MICROEXUDATE COATED FLASKS.

Microexudate coated flasks were prepared as previously described (Ackerman & Douglas 1978). Hep-2 cells were grown to confluence in tissue culture flasks (Sterilin, 75cm2) in MEM + 10% F.C.S. for 3 to 4 days. The culture medium was decanted, replaced with 10 to 15 mls of 10mM EDTA and incubated for 15 to 20 minutes. The detached cells were then decanted, the flasks thoroughly rinsed

with HBSS and inspected to ensure complete removal of all cells. The flasks were stored at 4°C until ready for use.

PURIFICATION OF MONOCYTES

After separation of mononuclear cells from 20mls of venous blood the cells were resuspended in 10mls of MEM + 20% F.C.S. and transferred to a microexudate coated flask.The flask was incubated for 1 hour at 37°C, following which nonadherent cells were washed out with three, 10ml aliquots of warm (37°C) MEM + 20% FCS. The adherent monocyte rich population was recovered by 20 minutes further incubation at 37°C with 2.0mls of 10mM EDTA and 5mls MEM + 20%FCS. The cells were then washed out with three, 10ml aliquots of MEM + 20% F.C.S., centrifuged at 200g for 10 minutes at 20°C and the pellet resuspended in 3mls MEM + 20% AB serum.

SECTION III: PREPARATION OF PHAGOCYTIC PARTICLES

1.0 PREPARATION OF YEAST

PREPARATION OF CANDIDA ALBICANS

C. albicans was grown aerobically in nutrient broth with 1% glucose at 37°C for three days, washed three times in normal saline and killed by heating at 90°C for 40 minutes.The killed yeast were washed three times in saline

PREPARATION OF SACCHAROMYCES CEREVISIAE (BAKERS YEAST)

Commercial bakers yeast were suspended in saline and dispersed by repeated syringing through a hypodermic needle. The yeast were killed by heating at 90°C for 40 minutes, then washed three times in saline.

SECTION IV: MEASUREMENT OF RATES OF PHAGOCYTOSIS

1.0 GENERAL CONSIDERATIONS AND DESIGN OF METHOD.

It was decided that any method chosen for the study of rates of phagocytosis by monocytes should meet the following requirements.

i) Phagocytosis by monocytes should be measured in cells in suspension rather than in adherent cell monolayers to avoid artificial selection of more activated subpopulations of cells.

ii) To avoid in vitro activation and alteration of functional properties which might mask the true in vivo functional state of monocytes, the method should use cells freshly obtained from subjects and long incubations should be avoided.

iii) The method, while following kinetic principles, should also be reasonably rapid and appropriate for use in a clinical setting.

2.0 MEASUREMENT OF RATES OF PHAGOCYTOSIS USING A DIRECT MICROSCOPIC METHOD.

Monocytes were separated from 20mls of venous blood using microexudate coated flasks as described above. The cells were resuspended to 10^6/ml in Eagles MEM + 20% AB serum.

Heat killed C. albicans were used as the phagocytic particle and were resuspended to make a stock suspension of $2x10^6$/ml.

The phagocytic assay was carried out as follows. 0.1mls of cell suspension, 0.3mls of MEM + 20% AB serum and 0.1ml of yeast suspension were added to screw topped plastic tubes (NUNC, 3.0ml). Pairs of tubes were incubated for 15, 30, 60 and 120 minutes in a shaking water bath at 37°C. Each tube was mixed thoroughly and an aliquot of suspension used to prepare a cytocentrifuge slide. The slides were stained with Jenner-Giemsa and mounted under coverslips. To assess phagocytosis at each time interval the number of intracellular yeast in 100 monocytes, and the percentage of monocytes actively phagocytosing were counted under the light microscope.

3.0 MEASUREMENT OF CELL LOSSES FROM SUSPENSION DURING INCUBATION.

Using the above method it was apparent that after incubation of cells at 37°C there was rapid loss of cells from suspension and therefore incomplete recovery of monocytes for assessment of phagocytosis.

To quantify cell losses from suspension during incubation, mononuclear cells were incubated as before, but without the addition of yeast. At timed intervals the supernatant was sampled and the number of cells in suspension counted using the Coulter counter. After 90 minutes of incubation 0.5mls of 5mM EDTA was added to each tube and the tubes incubated for a further 30 minutes before sampling the supernatant again.The incubations were performed either in plastic NUNC tubes (3.0ml), siliconised glass tubes (5.0ml), moulded Teflon (PTFE) (5.0ml) tubes or in round bottomed wells machined from a small block of Teflon (Klinger plastics). The volume of each well was 500ul, and diameter 8mm.

In some experiments the Coulter Channeliser was used to determine the relative numbers of lymphocytes and monocytes in suspension at each time interval.

4.0 MEASUREMENT OF RATES OF PHAGOCYTOSIS USING AN INDIRECT MICROSCOPIC METHOD.

Mononuclear cells were separated from 20mls of venous blood as described and resuspended in 1ml of HBSS+10%AB serum to an approximate final concentration of 10^7 monocytes/ml unless otherwise indicated. The exact concentration of monocytes was determined using the Coulter Counter as described. Heat killed C. albicans were resuspended in HBSS+0.1% gelatin to a final concentration of $7.5x10^7$/ml. 300ul of mononuclear cells and 20ul of yeast suspension were incubated in the well of a Teflon block at 37°C on a turntable at 20rpm, canted at 45degrees. 40ul of cell suspension were removed at 0, 15, 30 and 60 minutes, diluted in 200ul of counting fluid and the number of yeast remaining extracellular counted in two Neubauer haemacytometers.

5.0 INVESTIGATION OF ERRORS DUE TO VARIATION IN HAEMACYTOMETER COUNTS.

In the preliminary experiments it was found that there was considerable variation in yeast counts between individual haemacytometers and this was clearly a potential source of error in the measurement of rates of phagocytosis. To determine the number of haemacytometer counts which should be performed to minimise errors due to inter-haemacytometer variation the following experiment was conducted. 150ul of yeast suspension and 150ul of HBSS were dispensed into the six wells of a Teflon block without mononuclear cells. 100ul of yeast were removed from each well, diluted in a single 10mls aliquot of counting fluid, and the suspension loaded into one chamber of each of twelve double-chamber haemacytometers. The procedure was then repeated and the opposite chamber of each haemacytometer loaded. To determine the number of haemacytometers required to obtain an identical mean count from opposite chambers, 12 separate counts were performed and the number of yeast counted in each haemacytometer chamber recorded separately. The experiment was performed six times.

6.0 ELECTRON MICROSCOPIC STUDIES ON PHAGOCYTOSED YEAST

To obtain qualitative confirmation that yeast phagocytosis was occurring, electron microscopic studies on mononuclear cells were carried out after incubation of mononuclear cells with yeast for 5 and 10 minutes using the assay conditions described above. After incubation the cells and yeast were diluted with cold fixative (5% glutaraldehyde in 0.1M sodium cacodylate buffer pH7.2) and then pelleted by centrifugation. The pellet was washed three times in 0.3M sucrose in 0.1M sodium cacodylate buffer and then in 0.05M sodium cacodylate with 1% osmium tetroxide for 1 hour at 4°C. The pellet was then given three, 5 minute washes in each of 10% ethanol, absolute ethanol and epoxypropane. Following this, the pellet was mounted in araldite and allowed to set at 40°C prior to the preparation of thin sections for electron microscopy.

7.0 MEASUREMENT OF PHAGOCYTIC RATE CONSTANT USING PREOPSONISED YEAST.

METHOD FOR MEASUREMENT OF PHAGOCYTIC RATE CONSTANT IN CONTROLS AND PATIENTS

Mononuclear cells were separated from 10mls of venous blood and resuspended in approximately 1.3mls HBSS+0.1% gelatin giving a final concentration of between 2.0 and 4.0×10^6 monocytes/ml. The exact concentration of monocytes was determined on the Coulter Counter. 150ul of monocyte suspension and 150ul of preopsonised yeast (vide infra) were dispensed into the six wells of a Teflon block. 100ul aliquots were removed immediately after mixing and all six aliquots added to a single 10mls aliquot of counting fluid. After 20 minutes incubation at 37°C on a turntable a further 100ul aliquot was removed from each well and added to a second 10mls aliquot of counting fluid. The number of yeast remaining extracellular at the two time points were counted in opposite sides of twelve haemacytometers.

The phagocytic rate constant "K" was calculated from:

$$K = (1/t/Mo) \times \ln(Yo/Yt) \quad \text{mls/min/monocyte,}$$

where t = minutes, Mo = monocyte/ml, Yo and Yt are the extracellular yeast concentrations at 0 and t minutes respectively.

PREPARATION OF PREOPSONISED YEAST PARTICLES

The optimum ratios of yeast to opsonins were established by dose response control studies described in the results.

OPSONISATION OF CANDIDA ALBICANS WITH HUMAN SERUM

0.1ml of heat killed C. albicans ($7x10^7$ yeast/ml) were preopsonised prior to each experiment in 2.5mls of human AB serum at 37°C for 30 minutes. The opsonised yeast were then washed in excess HBSS and resuspended in 1ml of HBSS+0.1% gelatin giving a final concentration of between 1.0 and $1.5x10^7$/ml.

OPSONISATION OF CANDIDA ALBICANS WITH POOLED HUMAN IgG

Heat killed C. albicans were opsonised in bulk with purified human IgG (S.E.Scotland & Edinburgh Blood Transfusion Service) in a ratio of 10^8 C. albicans/50mg IgG/2mls of PBS. Opsonisation was carried out at 37°C for 60 minutes in plastic universal containers (Sterilin), following which the yeast were washed twice in HBSS+0.1% gelatin, resuspended to 10^7/ml and stored in 1ml aliquots in liquid nitrogen until ready for use.

This particle was used for measurement of Fc receptor mediated phagocytosis.

OPSONISATION OF SACCHAROMYCES CEREVISIAE WITH HUMAN SERUM

Heat killed S. cerevisiae were opsonised in bulk with fresh normal human serum from a single donor in a ratio of $5x10^8$ yeast/20mls serum. Opsonisation was carried out at 37°C for 30 minutes in plastic universal containers (Sterilin), following which the yeast were washed twice in HBSS+0.1% gelatin, resuspended to

10^7/ml and stored in 1.0ml aliquots in liquid nitrogen until ready for use.

This particle was used for measurement of "complement" receptor mediated phagocytosis.

CONTROL STUDIES ON THE TYPE OF OPSONINS PRESENT ON YEAST PARTICLES

In these experiments preopsonised yeast (serum treated S. cerevisiae or IgG treated C. albicans) were incubated with F(ab')2 fragments of IgG anti-complement and anti-Fc antibodies (American Hospital Supplies) prior to offering the yeast for ingestion in the direct phagocytic assay. The inhibitory effects of F(ab')2 anti-Clq, C4, C3/C3c, βlH and Fc on phagocytosis were then studied.

Preparation of F(ab')2 fragments:

F(ab')2 fragments of IgG antibodies were prepared as previously described (Nisonoff et al 1960). The IgG was dialysed against several changes of acetate buffer (0.07M acetate buffer pH4.0 in 0.05M sodium chloride) for 24 hours. The IgG was incubated with pepsin (3mg pepsin/100mg protein) in a water bath at 37°C for 18 hours. The pH was then adjusted to 8.0 with 1N NaOH and dialysed against several changes of borate buffered saline pH 8.0. The F(ab')2 fraction was further purified on a Sephadex G150 column, and concentrated by pervaporation. The protein concentration was obtained from the absorbance at 280mu using an extinction coefficient of 13.8.

8.0 MEASUREMENT OF PERCENTAGE OF PHAGOCYTIC CELLS IN MIXED MONONUCLEAR CELL POPULATIONS USING A DIRECT MICROSCOPIC METHOD.

To assess the percentage of "complement" receptor and Fc receptor positive phagocytic cells in a mixed mononuclear cell (lymphocytes and monocytes) suspension the direct microscopic method of measuring phagocytosis (Section 2.0) was used in modified form.

Mononuclear cells were separated from 10mls of venous blood and resuspended in approximately 1.3mls HBSS+0.1% gelatin giving a concentration of between 2.0 and 4.0×10^6 monocytes/ml. The exact concentration of cells was determined both by Coulter sizing and NSE staining. 150ul of monocyte suspension and 150ul of either complement coated S. cerevisiae or IgG coated C. albicans were dispensed into the wells of a Teflon block, and incubated at 37°C on a turntable, at a 45 degree cant, rotating at 12RPM. After 40 minutes of incubation a 100ul aliquot was removed diluted in 10mls of cold HBSS+0.1% gelatin and cytocentrifuge slides prepared. The slides were stained with nonspecific esterase and the percentage of phagocytic cells was estimated microscopically from a count of at least 200 mononuclear cells.

9.0 IN VITRO CULTURES OF MONONUCLEAR CELLS

For experiments in which mononuclear cells were studied in monolayers in vitro the following procedure was used. Mononuclear cells were suspended in RPMI 1640 supplemented with 20% fetal calf serum (heat inactivated 56°C for 60 minutes), glutamine (20mM), penicillin and streptomycin. 1.0-2.0mls of mononuclear cell suspension (10^6/ml) were plated onto glass coverslips in round flatbottomed plastic culture dishes (Linbro, 12 well, 2.4x1.7cms, capacity 7.5mls), and incubated in 5%CO2/air at 37°C. After one hour the nonadherent cells were washed off with 2 or 3 aliquots of warm media.

Monolayers were assessed at different times for %age of phagocytic and NSE positive cells. To determine the %age of phagocytic cells duplicate coverslips were overlaid with either "complement" or IgG coated yeast (0.5-1.0mls of stock suspension) for 40 minutes, the coverslips washed gently to remove excess yeast and then stained with NSE/methyl green. A count of at least 200 cells was performed to determine the %age of phagocytic cells.

SECTION V . METHODS FOR DETECTING AND MEASURING SERUM COMPLEMENT, IMMUNE COMPLEXES AND AUTOANTIBODIES.

1.0 STORAGE OF SERUM AND EDTA PLASMA.

Serum was separated from whole blood which had been clotted at room temperature for 20-30 minutes. The serum was then stored immediately in 0.5ml aliquots in liquid nitrogen until ready for use.

EDTA plasma was obtained by collecting 4mls of whole blood into potassium EDTA tubes (Brunswick BS4851) and centrifugation at room temperature. Plasma was stored in 0.5ml aliquots in liquid nitrogen until ready for use.

2.0 MEASUREMENT OF SERUM C3 AND C4.

Serum C3 and C4 were measured by radial immunodiffusion using commercially available kits (Seward Laboratories Immunostics).

3.0 MEASUREMENT OF SERUM ANTICOMPLEMENTARY ACTIVITY.

Test sera are incubated with guinea-pig serum (GPC) and extent of complement consumption assayed by measurement of reduction in haemolytic activity using sensitised erythrocytes (RBC). Complement fixation diluent (CFT) is used throughout as buffer.

Sensitised erythrocytes (EA) are prepared by incubating RBC at 37°C with a predetermined subagglutinating concentration of anti-SRBC haemolysin, followed by two washes and dilution to give a 10% suspension.

GPC is reconstituted with 2ml of distilled water and allowed to stand for 15 minutes, following which the solution is diluted to 1/10 with 18mls of CFD at 4°C.

The GPC is next titrated to determine the highest dilution which causes complete SRBC lysis. Dilutions of GPC are prepared by dilution with GPC (1/10, 1/20, 1/30...1/100). 50ul of each dilution is transferred to a "U" microtitre plate. 50ul of CFD and 50ul 2.5% EA are added to each well, mixed and incubated at 37°C for one hour, with further agitation after 30 minutes. The plate is spun at 1,000rpm for 5 minutes. The highest dilution of GPC causing complete EA lysis is recorded (100 H.D.).

Test sera, including a positive and negative control, are taken from liquid nitrogen and heat inactivated for exactly 30 minutes at exactly 56°C.

To perform the anti-complementary assay, 50ul of CFD is added to each test well followed by 50ul of heat inactivated serum to well number one. Using a 50ul microdilutor, the samples are double

diluted to well twelve. Next 50ul of CFD and 50ul of 2X100 H.D. are added and the wells mixed and incubated at 4°C overnight. 50ul 2.5% EA are added to all wells, mixed and incubated at 37°C for 1 hour. The plates are shaken again after 30 minutes. The plates are then spun for 5 minutes at 1000rpm.

Each well is scored according to the extent of EA lysis on a scale (100% lysis = 0; 75% lysis = +1; 50% lysis = +2; 25% lysis = +3; No lysis = +4). The anticomplementary titre is the last dilution of serum exhibiting 50% lysis (i.e. a score of +2).

4.0 MEASUREMENT OF SERUM Clq BINDING ACTIVITY.

PURIFICATION OF Clq

Clq was prepared according to the method of Yonemasu and Stroud (1971).

The following buffers are used:

0.026M EGTA pH7.5

0.06M EDTA pH5.0

0.75M NaCl, 0.02M acetate, 0.01M EDTA pH5.0

0.75M NaCl, 0.02M acetate, 0.01M EDTA pH7.5

400mls of blood was obtained from a starved volunteer and collected into 2 blood bottles treated with Repelcote and filled with paper clips. The blood was then defibrinated, the serum separated and centrifuged at 10,000g (J2.21, angle head rotor, 15ml Corex glass tubes, 9,000 rpm) at 4°C for 20 minutes.

The surface layer of lipid is removed and discarded. The volume of serum is noted and a 0.5ml aliquot saved.

The serum is dialysed against 0.026M EGTA pH7.5: 1l. for 1 hour, 2l. for 2 hours, 2l. overnight at 4°C. The dialysed serum is centrifuged at 10,000g for 20 minutes at 4°C in 30ml Corex glass tubes, the supernatant removed, a small aliquot saved and the remainder discarded. The precipitate is washed with 30ml 0.026M EGTA pH7.5, and the centrifugation repeated. Again the supernatant is discarded except for a small aliquot.

The precipitate is creamed into 0.75M NaCl, 0.02M acetate buffer containing 0.01M EDTA pH5.0. The redissolved protein is

pooled to a final volume of 30ml, centrifuged at 5,000g at 4°C for 20 minutes, and the supernatant decanted. An aliquot is saved.

The supernatant is dialysed against 0.06M EDTA pH5.0: 1l. for one hour, 2l. for one hour, 2l. for two hours at 4°C. The dialysate is centrifuged at 10,000g for 20 minutes at 4°C, the supernatant removed, an aliquot saved and the remainder discarded. The precipitate is washed with 30ml 0.06M EDTA, and centrifuged at 10,000g for 20 minutes at 4°C. The supernatant is removed, an aliquot saved and the remainder discarded. The precipitate is creamed into 15ml of 0.75M NaCl in 0.02M acetate buffer containing 0.01M EDTA pH7.5. The resolubilised protein is centrifuged at 5,000g for 20 minutes at 4°C, and the supernatant decanted avoiding insoluble aggregates. The supernatant is dialysed overnight at 4°C against 5 litres of PBS. The C1q is stored in 1ml aliquots in liquid nitrogen.

ANALYSIS OF FRACTIONS

Each fraction is analysed by radial immunodiffusion (RID). For analysis of immunoglobulin fractions, buffer A (Tris hydroxymethyl methyl amine 2.42g + glycine 6g + disodium 3.72g + NaCl 8.78g to 1 litre) is used and for Clq, buffer B (sodium barbitone 9.3g + barbitone 1.3g to 1 litre) is used.

Protein to be estimated	Buffer	Antibody (DAKO)	Volume of antibody	Dilution of normal serum for standard
IgA	B	A092	170ul	1/1, 2, 4, 8, 32.
IgG	B	A090	55ul	1/16, 32, 64, 128, 512
IgM	B	A091	55ul	1/1, 2, 4, 8, 32.
Clq	A	A136	10ul	1/1, 2, 4, 8.

Radial immunodiffusion plates are prepared with 10ml of Seakem ME agarose (1%) per plate, a well diameter of 3mm and a sample volume of 5ul. The plates are incubated in a humid atmosphere for 48 hours, washed, pressed and stained.

The protein concentration of the Clq fraction is obtained from the optical density at 280nm (OD280 at 1mg/ml is 0.682).

PREPARATION OF RADIOLABELLED Clq

Reagents are prepared on the day of use and kept at 4°C in iced water:

1) Lactoperoxidase 5mg (SIGMA) - 100ul + 400ul PBS

2) Hydrogen peroxide 30% w/v - 2ul + 10ml PBS

3) 2-mercaptoethanol 3.5ul + 10ml PBS

4) Potassium iodide 10mg + 10ml PBS

An aliquot of Clq is thawed rapidly at 37°C in a water bath, transferred to a glass tube and placed in ice. The following reagents are then added in order:
50ul of lactoperoxidase, 500 uCi (5ul) of IMS-30 (125)iodine, 50ul of hydrogen peroxide, mixed well and incubated at 4°C for 15 minutes. Then add 2.5ml of 2-mercaptoethanol and 250ul of potassium iodide and mix well. The reaction mixture is transferred to visking tubing and dialysed overnight against PBS.

The radiolabelled Clq is diluted with 1%BSA/PBS to a final volume of 10ml. Aliquots are stored in the vapour phase of liquid nitrogen.

125-IODINE Clq BINDING ASSAY FOR IMMUNE COMPLEXES

In the assay, test sera are incubated with purified radiolabelled Clq; immune complexes binding to Clq are selectively precipitated from solution with polyethyleneglycol.

The following reagents are required:

1%BSA/PBS - 0.5mls 20% BSA + 9.5ml PBS; 0.2M EDTA pH7.5; 0.1M borate buffer pH8.3.

An aliquot of labelled Clq in 1%BSA/PBS is transferred to a 15ml glass Corex tube and centrifuge at 18,000g (J2-21, 16,000 rpm) for 1 hour at 4°C. 50ul test serum and 100ul 0.2M EDTA are added to duplicate LP3 tubes, mixed and incubated for 30 minutes at 37°C. The tubes are transferred to an ice bath, 50ul of the spun Clq added to each and mixed. 1ml of 3% PEG in borate buffer is added immediately and the tubes incubated on ice for 1 hour. The tubes are centrifuged at 1500g for 20 minutes at 4°C, and the supernatant decanted. Each tube is capped and then counted in a gamma counter (minimum count 10,000 in every tube). A control tube of known Clq % binding activity is assayed in each run and unknowns are calculated in relation to this control. Duplicates varying by more than 10% are repeated. (Normal range 0-20%).

5.0 MEASUREMENT OF RHEUMATOID FACTOR TITRES.

Rheumatoid factor titres were measured by agglutination of human erythrocytes (RBC) sensitised with subagglutinating titres of sheep IgG anti-human group O, Rhesus negative RBC (Pudliachouk et al 1958). All sera were heat inactivated at 56°C for 30 minutes and tested at doubling dilutions (lowest 1/20) on an agglutination plate. Occasional control wells showed agglutination of unsensitised RBC and in these cases the sera (1/20) was adsorbed with an equal volume of unsensitised RBC for 1 hour at 37°C before retesting.

6.0 MEASUREMENT OF ANTI-NUCLEAR ANTIBODY TITRES.

Antinuclear antibodies were detected by indirect immunofluorescence using human blood polymorphonuclear neutrophils as substrate (Alexander et al 1960). Smears of human capillary blood were made on methanol cleaned glass slides, fixed in 95% ethanol for 15 minutes and air dried for 15 minutes.

Test serum samples (minimum dilution 1/20) and positive and negative controls were placed on marked areas of the smear and incubated at room temperature for 30 minutes in a damp chamber.

Slides are washed X3 in PBS, following which swine anti-human gamma-globulin (FITC) (1/20) is added and the slides further incubated for 30 minutes. The slides are washed X3 in PBS and examined under PLOEM illumination for nuclear fluorescence.

7.0 DETECTION OF PRECIPITATING ANTIBODY TO SALINE SOLUBLE CELLULAR ANTIGENS.

Antibodies to Sm and nRNP were detected using human spleen extract and antibodies to Ro and La using calf thymus extract (Mattioli & Reichlin 1971).

Fresh human spleen extract was homogenised in PBS, centrifuged to remove debris and the soluble extract dialysed against 0.01M phosphate buffer pH7.2 for 6 hours at 4°C. After dialysis the extract was equilibrated with with DE52 gel in 0.01M phosphate buffer pH7.2 at 4°C. The gel was vacuum filtered and washed with 0.01M phosphate buffer pH7.2 at 4°C. The gel was then placed in 0.15M NaCl/0.01M phosphate pH7.2 at 4°C overnight with stirring. The gel was again filtered at the Buchner and then placed in 0.5M NaCl/0.01M phosphate buffer pH7.2 overnight with stirring. The gel was filtered and the filtrate collected and centrifuged to remove any DE52 particles and then concentrated by pervaporation. After concentration, the extract was dialysed against 0.15M Na/Cl/0.01M phosphate buffer pH7.2 overnight. The extract was then used directly in Ouchterlouny plates for detection of ant-Sm or anti-RNP antibodies using control sera showing a line of identity with sera obtained from Drs Tan and Reichlin.

Commercially available dried calf thymus extract (Pel-Freeze) was rehydrated with PBS, homogenised and centrifuged to remove debris. The soluble supernatant was concentrated by pervaporation.

7.0 MEASUREMENT OF ANTI-DNA BINDING ACTIVITY

Serum anti-DNA binding activity was performed using the Farr technique (Amersham Radiochemicals). These measurements were performed by Dr K.C. Watson, Dept of Bacteriology, Western General Hospital, Edinburgh.

9.0 STATISTICS.

Parametric and non-parametric statistics were applied as appropriate and the precise methods used are indicated where relevant in the text.

CHAPTER 6

RESULTS

SECTION I: PURIFICATION OF CELLS.

1.0 MONOCYTE YIELDS FROM FICOLL-HYPAQUE DENSITY GRADIENT AND MICROEXUDATE COATED FLASKS.

A pilot study of the method for mononuclear cell separation from 20mls of normal blood (n=7) (Table 6.1) showed that recovery of monocytes from a Ficoll-Hypaque gradient was greater than 90% and gave a mononuclear fraction comprising 20% monocytes and 80% lymphocytes. After further purification of adherent cells in microexudate coated flasks, a monocyte enriched fraction was obtained containing 85% monocytes but with reduction of overall monocyte recovery to 50%. The viability of cells assessed by trypan blue was greater than 94%.

Table 6.1 Mean monocyte yields (+/-SD) from density gradient and microexudate coated flasks (n=7).

FRACTION	TOTAL WBC	Mo %	Mo yield	Recovery
Whole blood	$120x10^6$ (+/-29)	6.1 (+/-2)	$7.1x10^6$	-
Mononuclear fraction	$32x10^6$ (+/-7)	20.4 (+/-5)	$6.5x10^6$	91.5%
Adherent cell fraction	$4.2x10^6$ (+/-2)	84.5 (+/-5)	$3.6x10^6$	50.1%

SECTION II: IDENTIFICATION OF MONOCYTES AND LYMPHOCYTES.

1.0 INTRODUCTION.

The size distribution of mixed mononuclear cells, purified lymphocytes, and purified monocytes were studied by performing cell counts within different "size window" settings on the Coulter counter ranging from 5.6 to 11.3 microns. Additionally some size distribution studies were performed using a "Coulter Channeliser" (loaned by the Coulter Company Inc). The latter enabled a continuous size-number distribution curve to be plotted on an X-Y plotter.

Numbers and percentages of monocytes and lymphocytes estimated by Coulter sizing were compared with NSE stains on (i) mixed mononuclear cells (ii) lymphocytes and monocytes purified in microexudate coated flasks.

2.0 SIZE DISTRIBUTION OF MONONUCLEAR CELLS AND IDENTIFICATION OF MONOCYTES BY VOLUME AND STAINING FOR NON SPECIFIC ESTERASE.

A typical size histogram of mononuclear cells obtained after Ficoll-Hypaque sedimentation is shown in Fig II.1A. The majority of mononuclear cells have a diameter greater than 6.2 microns. After depletion of adherent cells in microexudate coated flasks, the majority of large cells with diameter greater than 7.8 microns have been removed (Fig II.1B). Staining of the non-adherent cells with NSE confirmed that less than 5% were NSE positive monocytes. After recovery from the flasks, the adherent cells were found to be composed mainly of large cells with diameter greater than 7.8 microns (Fig II.1C) and 80% were NSE positive monocytes. The same result is graphically illustrated by use of the Coulter Channeliser which demonstrate the presence of two cell populations - one small and nonadherent, the other large and adherent; size distribution curves before and after depletion of adherent cells are shown in Fig II.2. The size distribution of adherent cells after recovery with EDTA is shown in Fig II.3

Differential counts of monocytes in mononuclear cell populations obtained from normal subjects determined by NSE staining (Fig II.4) correlated well with counts obtained by Coulter sizing in which lymphocytes were defined as cells of diameter 6.2 to 7.8 microns and monocytes were defined as mononuclear cells with diameter greater than 7.8 microns (i.e. volume >250 cub microns)(Fig II.5) ($n=19; r=0.78; p<0.001$).

Figure II.1 Size (cell diameter, microns) distribution histogram of mononuclear cells:

A - Mixed lymphocyte and monocyte population from Ficoll-Hypaque density gradient (20% NSE positive)

B - Mononuclear cells after depletion of adherent cells (<5% NSE positive)

C - Adherent cells after recovery from culture flasks (>80% NSE positive)

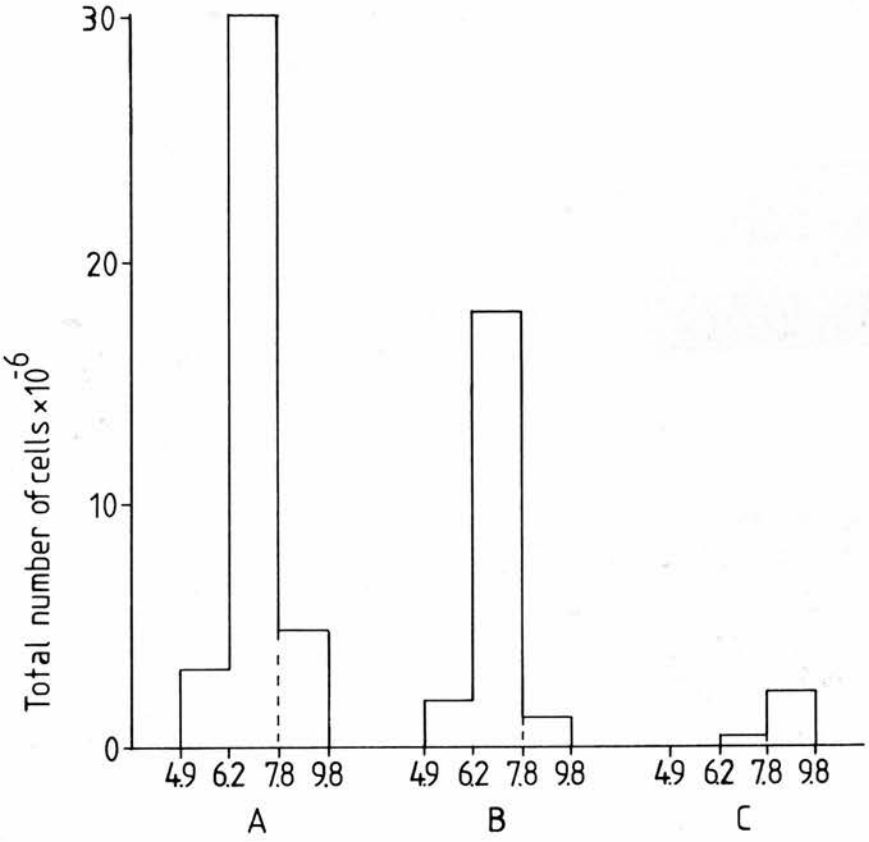


Figure II.2 Coulter "Channeliser" plot of size distribution of mononuclear cells before and after depletion of adherent cells:

("Channel number" represents arbitrary units of cell size)

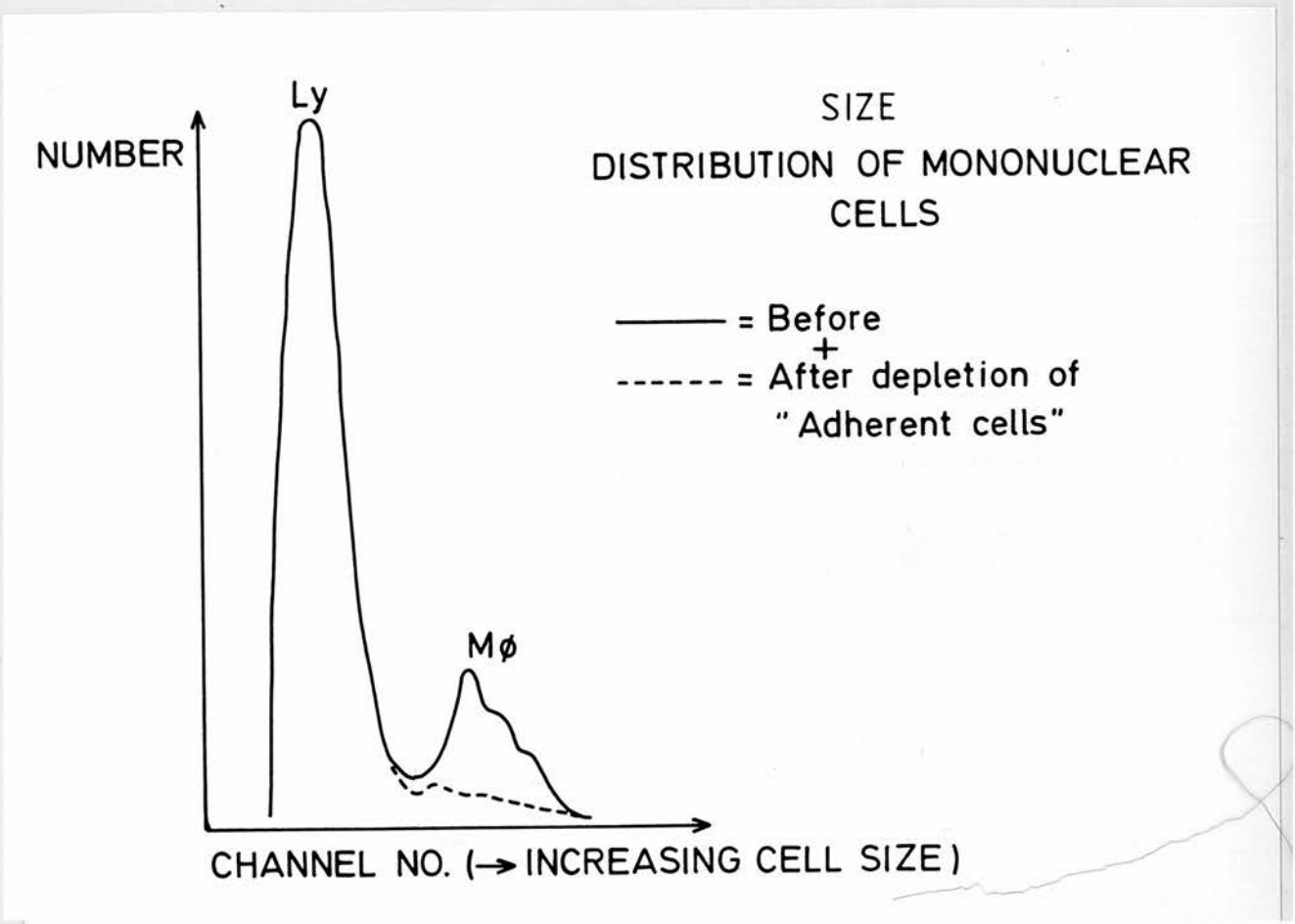


Figure II.3 Coulter "Channeliser" plot of size distribution of adherent mononuclear cells after recovery with EDTA:

("Channel size" represents arbitrary units of cell size)

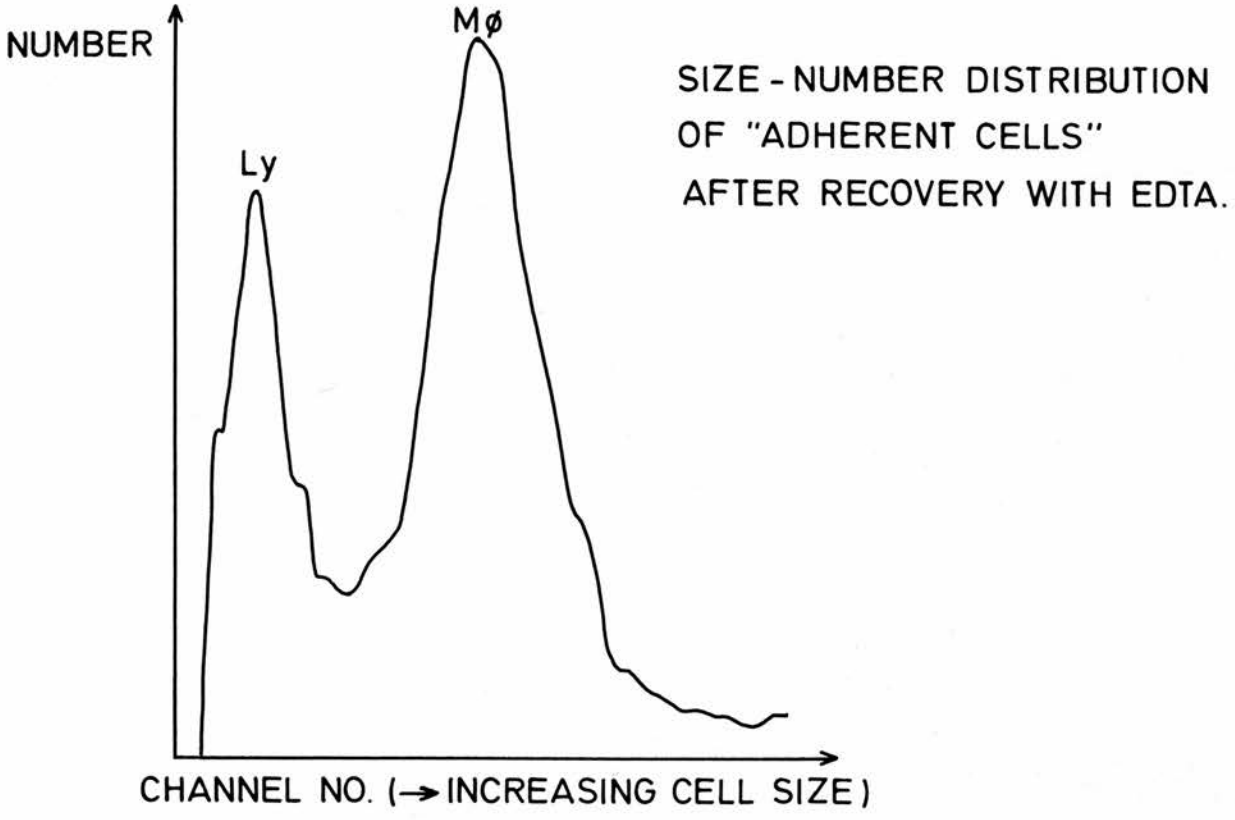


Figure II.4 Typical appearance of monocytes stained with nonspecific esterase (NSE) and alpha-chloroacetate esterase. Monocytes stain diffusely brown for NSE and occasionally have blue, paranuclear, chloroacetate-positive staining as well. Lymphocytes are counterstained with methyl green and some contain one or two NSE positive granules:

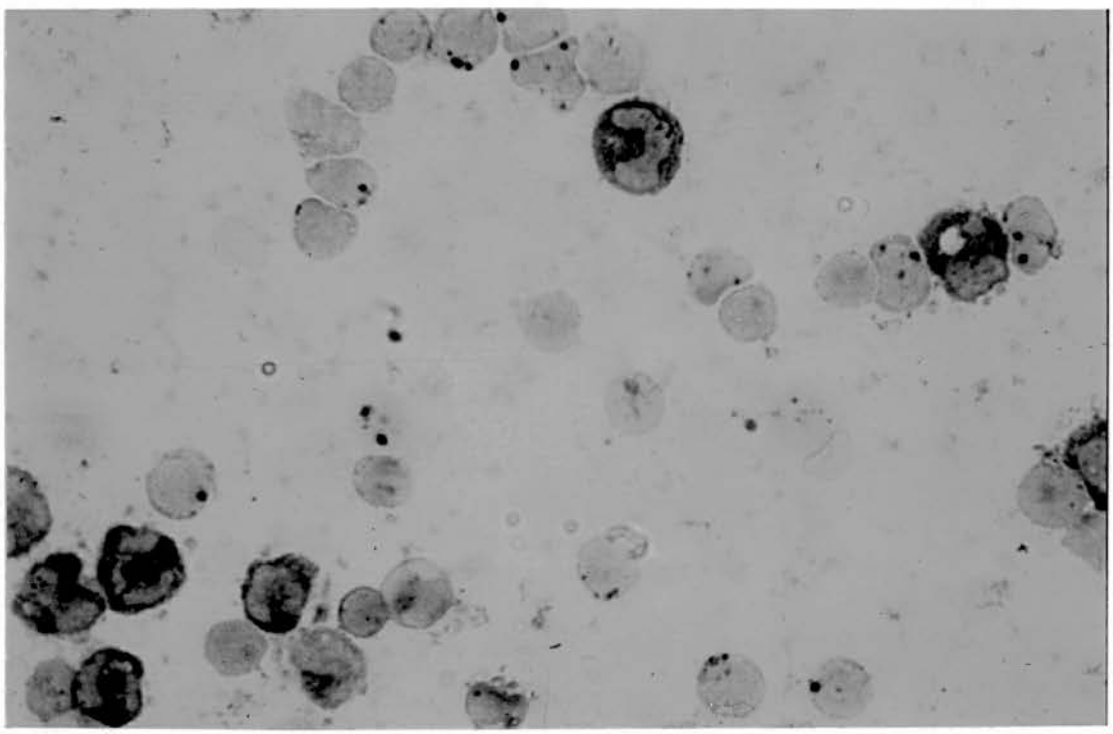
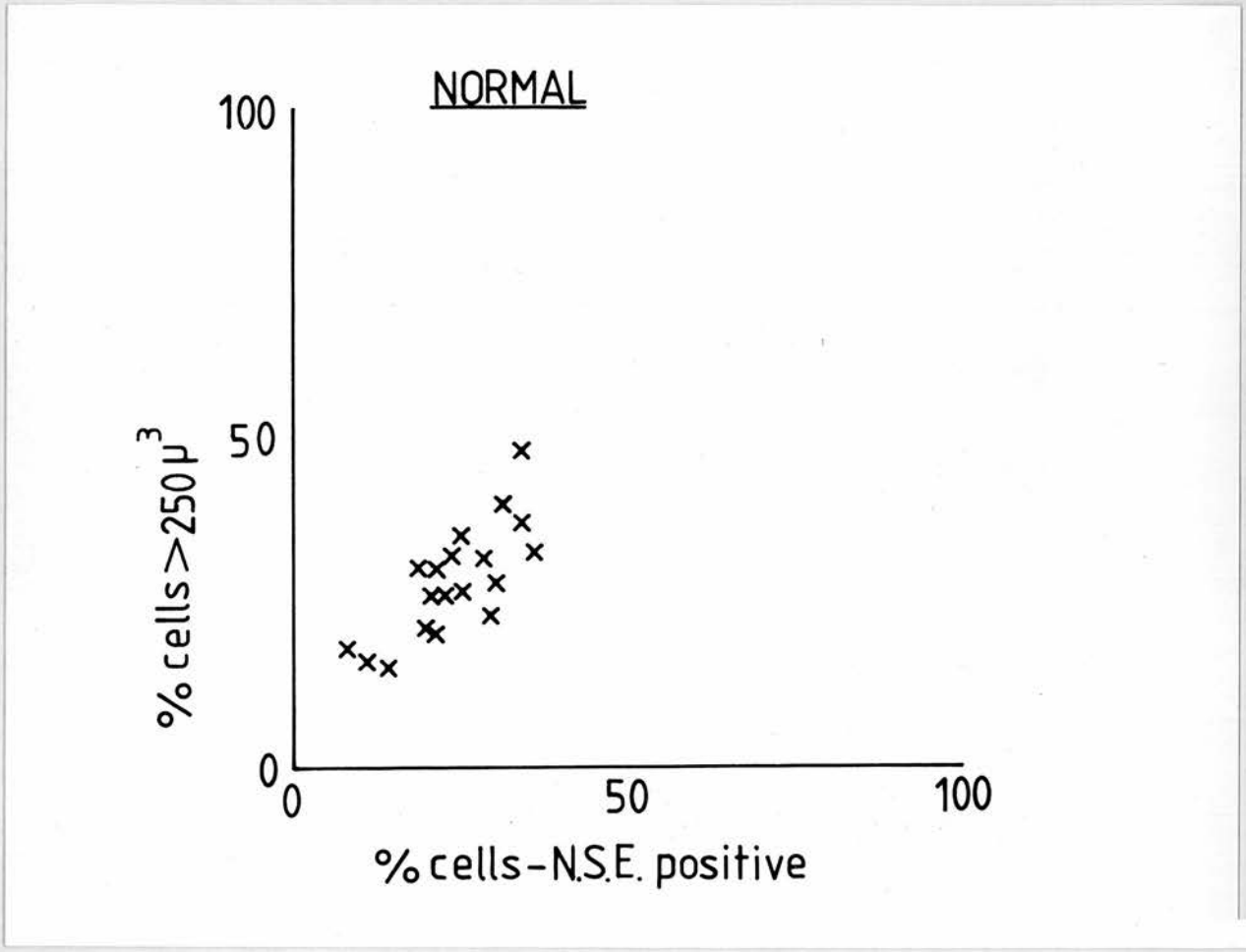


Figure II.5 %ages of monocytes in mixed mononuclear populations.

Differential counts obtained by NSE staining correlate well with the Coulter "sizing" method in which monocytes are defined as cells of volume greater than 250 cub microns (n=19;r=0.78;p<0.001):



SECTION III. RESULTS OF PHAGOCYTIC STUDIES - DEVELOPMENT OF METHODS AND CONTROL STUDIES

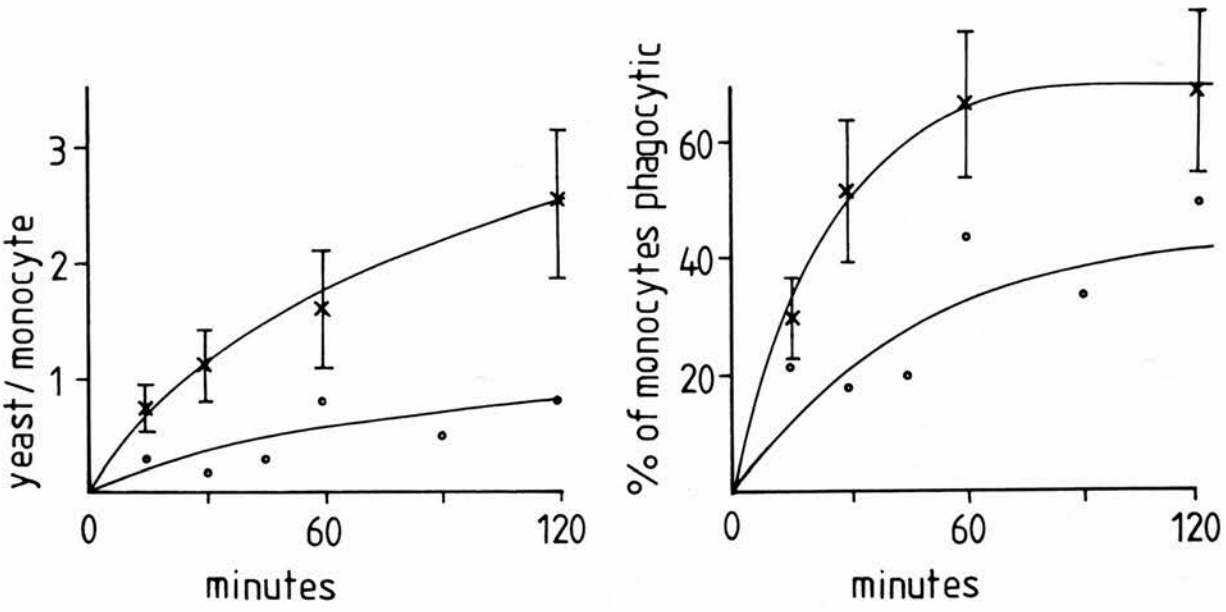
1.0 MEASUREMENT OF RATES OF PHAGOCYTOSIS USING DIRECT MICROSCOPIC METHOD.

TIME COURSE.

The method is described above (p190). The time course of phagocytosis of serum opsonised C. albicans over 120 minutes is shown in Fig III.1. Each point represents the mean (+/- s.e.m.) of three experiments. The mean (+/- s.d.) monocyte concentration was 0.2×10^6/ml (+/- 0.01) and mean yeast concentration 0.4×10^6/ml. The phagocytosis of unopsonised yeast was also studied (n=1); unopsonised yeast were phagocytosed, but at a very slow rate.

Fig III.1 Time course of phagocytosis of C. albicans by monocytes in presence (X) or absence (o) of 20% human AB serum.

Number of yeasts per monocyte and %age of phagocytosing monocytes are shown separately:



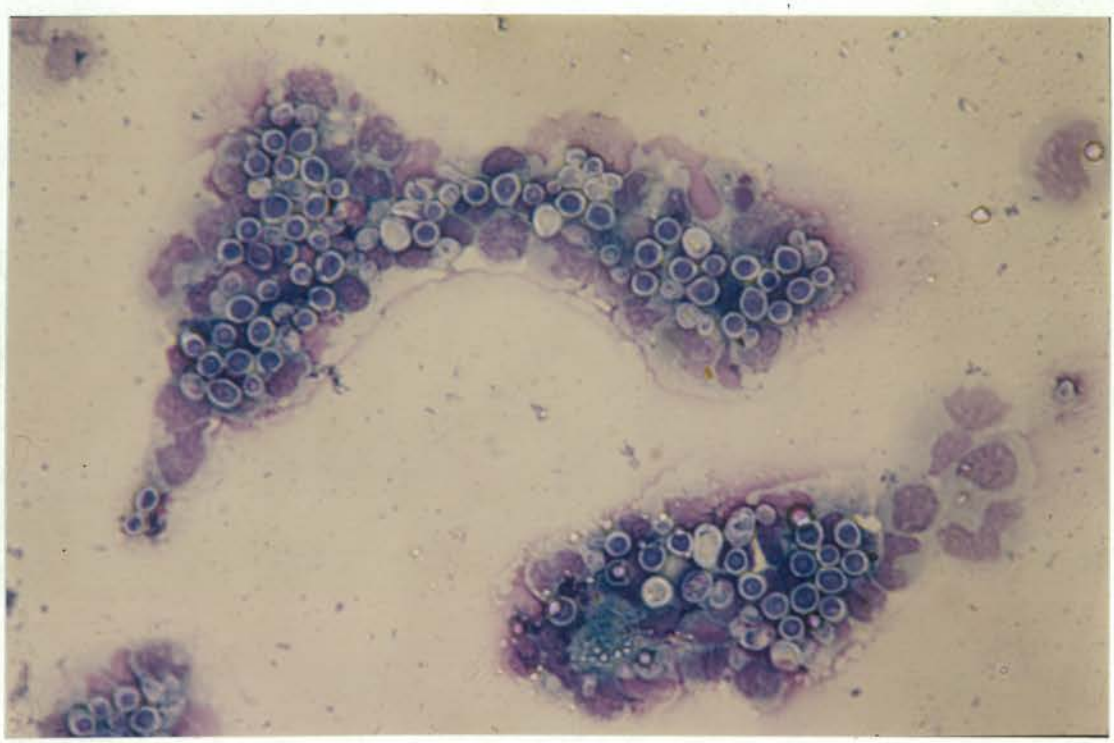
CRITICISM OF METHOD.

Several problems were apparent with this method. Firstly because of the formation of clumps of cells and yeast, particularly at later stages in the time course, it was difficult to determine whether yeast were ingested or not (Fig III.2). This may have been due in part to the generation of complement activation products by the action of yeast on fresh serum, and in later experiments using a direct microscopic method of assessing phagocytosis, preopsonised particles were used. Furthermore, calculation of the percentage of phagocytosing monocytes was liable to error because the Jenner Giemsa stain does not reliably distinguish between lymphocytes and monocytes and it was difficult to determine the number of monocytes present in clumps. The method was also time consuming and a single experiment, including cell separation, took approximately five hours. Finally it was apparent from the stained slides that there was rapid loss of mononuclear cells from suspension during incubation with yeast and therefore only incomplete recovery of monocytes for assessment of phagocytosis (vide infra). This was clearly a potential source of error in assessing rates of phagocytosis and percentages of phagocytic cells.

For these reasons alternative methods of assessing rates of phagocytosis were investigated. Additionally, since the loss of cells from suspension was a potential source of error in future experiments, this was investigated to see whether the problem could be minimised.

Fig III.2 Microscopic appearance of yeast and monocytes after 60 minutes of incubation in 20% AB serum.

Large clumps of monocytes and yeasts form during incubation making assessment of phagcytosis difficult:



2.0 RESULTS OF STUDY OF CELL LOSSES FROM SUSPENSION DURING INCUBATION.

After incubation of mononuclear cells without the addition of yeast, there was rapid loss of cells from suspension and by 30 minutes over 60% of cells had been lost (Fig III.3). After further incubation with EDTA, the cells were completely recovered, confirming that they had adhered to the walls of the container. An identical result was obtained with plastic tubes (NUNC, 3ml), siliconised glass tubes (5ml) and sintered Teflon tubes.

Using the Coulter channeliser, it was shown that these cell losses were due to selective depletion of monocytes (Fig III.4).

However, mononuclear cells incubated in wells machined from Teflon block remained in suspension for up to 60 minutes (Fig III.5), without selective depletion of monocytes. In all subsequent experiments mononuclear cells were incubated in the wells of a Teflon block.

Fig III.3 Cell losses due to adherence to incubation tube.

After incubation in a variety of plastic or siliconised containers, mononuclear cells were rapidly lost frm suspension, but could be recovered by further incubation with EDTA (2mM final). The diagram shows a representative experiment.

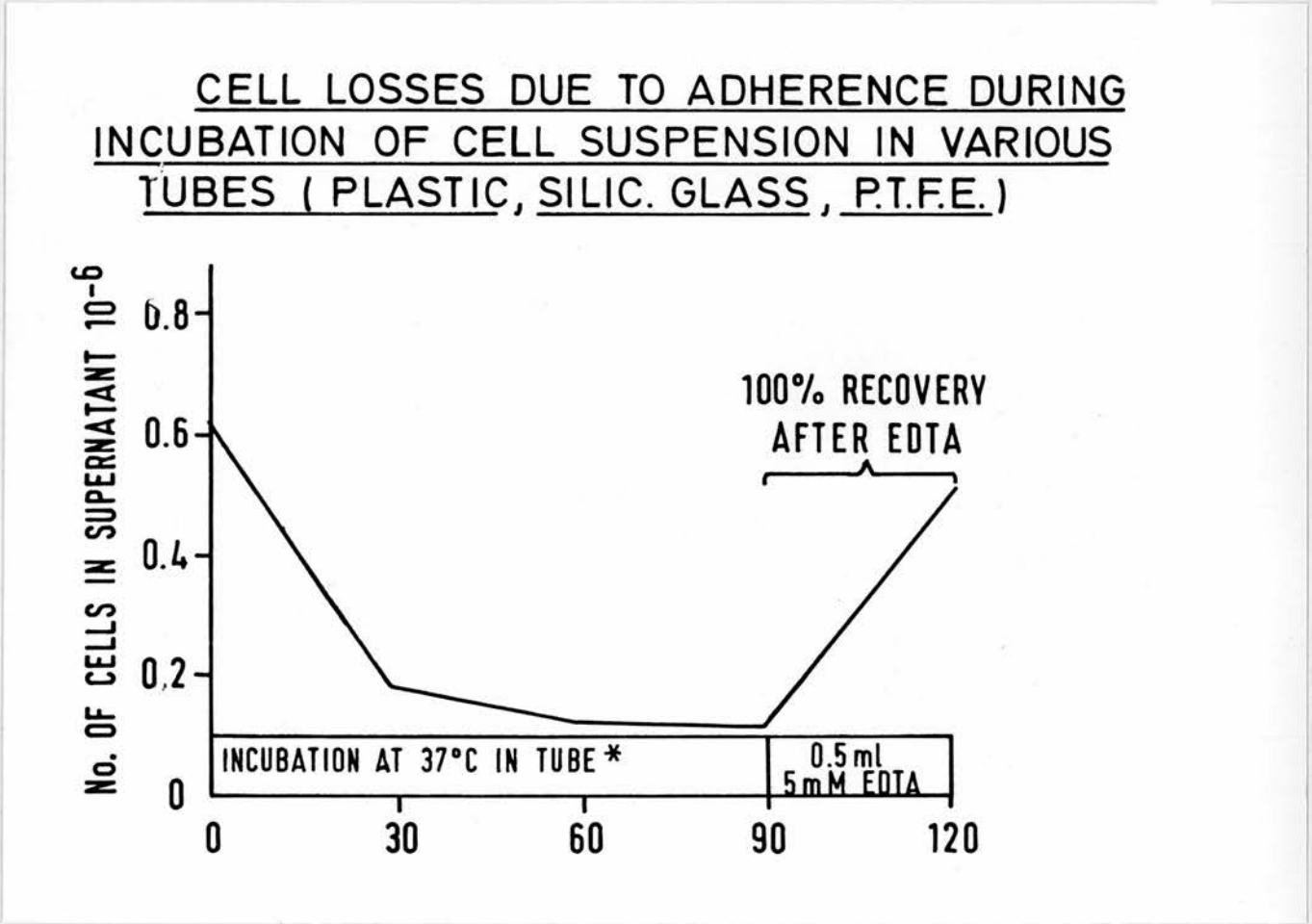
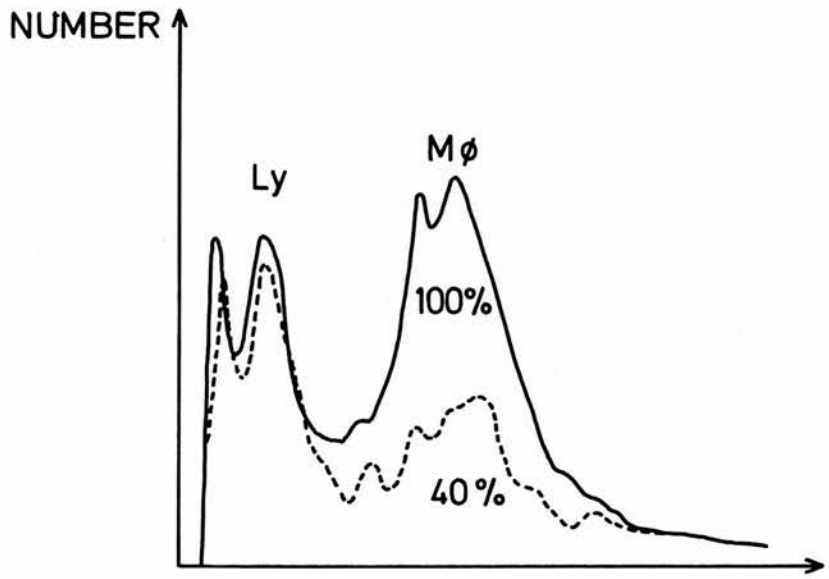


Fig III.4 Coulter "Channeliser" size distribution plot of mononuclear cells in suspension before (——) and after 30 minutes (----) incubation in plastic or glass tubes.

Cell losses were almost entirely attributable to loss of the large cell population (i.e. monocytes).

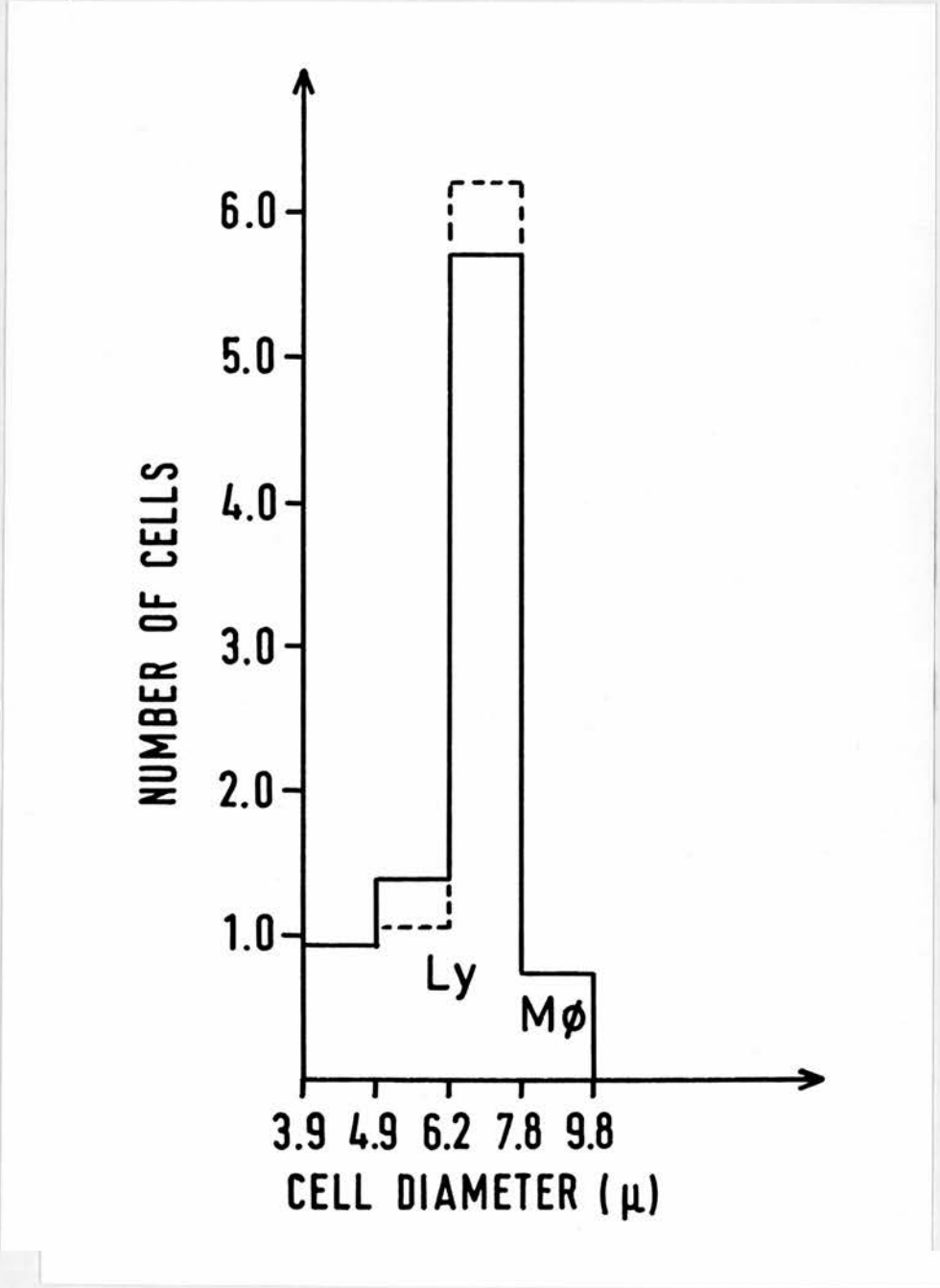
("Channel number" represents arbitrary units of cell size)



DISTRIBUTION OF A "MONOCYTE-RICH" POPULATION – SELECTIVE DEPLETION OF MONOCYTES AFTER INCUBATION AT 37°C

Fig III.5 Coulter size histogram of mononuclear cells before (——) and after 60 minutes (----) incubation in machined, round-bottomed Teflon wells (n=2).

No loss of monocytes (>7.8 micron diam) is seen.



3.0 MEASUREMENT OF RATES OF PHAGOCYTOSIS USING AN "INDIRECT" MICROSCOPIC METHOD - INITIAL CONTROL STUDIES.

INTRODUCTION.

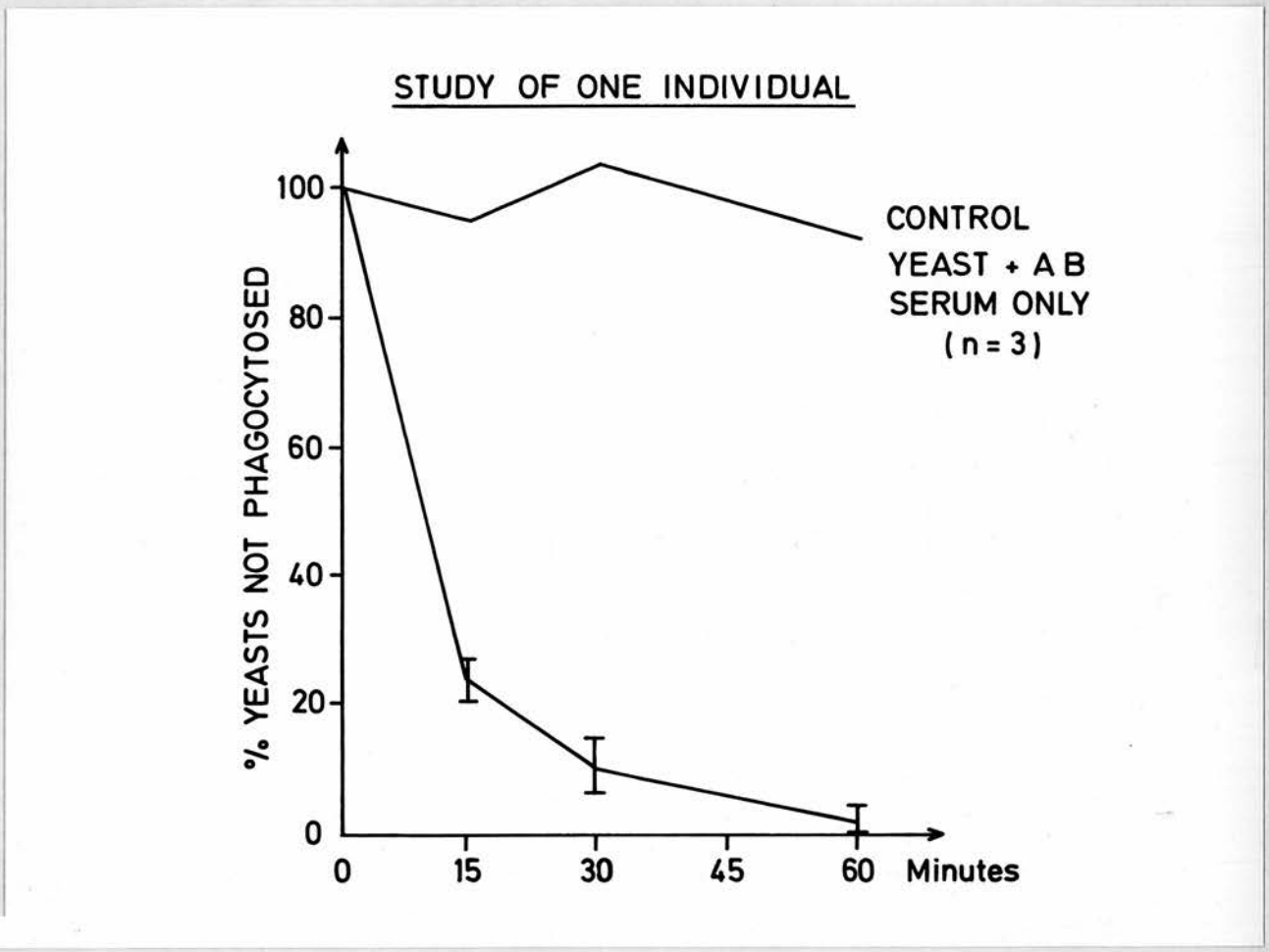
In view of the difficulties experienced in assessing rates of phagocytosis by direct microscopic evaluation of ingested yeast, an alternative "indirect" method described by Leijh et al (1977) was investigated. In this method, the uptake of yeast is followed by counting numbers of extracellular yeast in haemacytometers using diluted aliquots of yeast-monocyte suspension which have been incubated for timed intervals. No attempt is made to count ingested yeast particles. The method is described on p193.

TIME COURSE IN ONE INDIVIDUAL.

Using this "indirect" method the time course of phagocytosis was measured using mononuclear cells from one individual on 12 separate occasions.

The time course of phagocytosis is shown in Fig III.6. Disappearance of yeast from suspension is rapid and is expressed as the %age (+/-s.d.) of yeast remaining extracellular at each time point. The mean (+/- s.d.) monocyte concentration was $5.39(+/-0.92)x10^{6}$/ml.

Fig III.6 Time course of phagocytosis by monocytes from one individual (n=12).



TIME COURSE IN NORMAL CONTROL SUBJECTS AND EFFECT OF N-ETHYL MALEIMIDE AND TEMPERATURE ON PHAGOCYTOSIS.

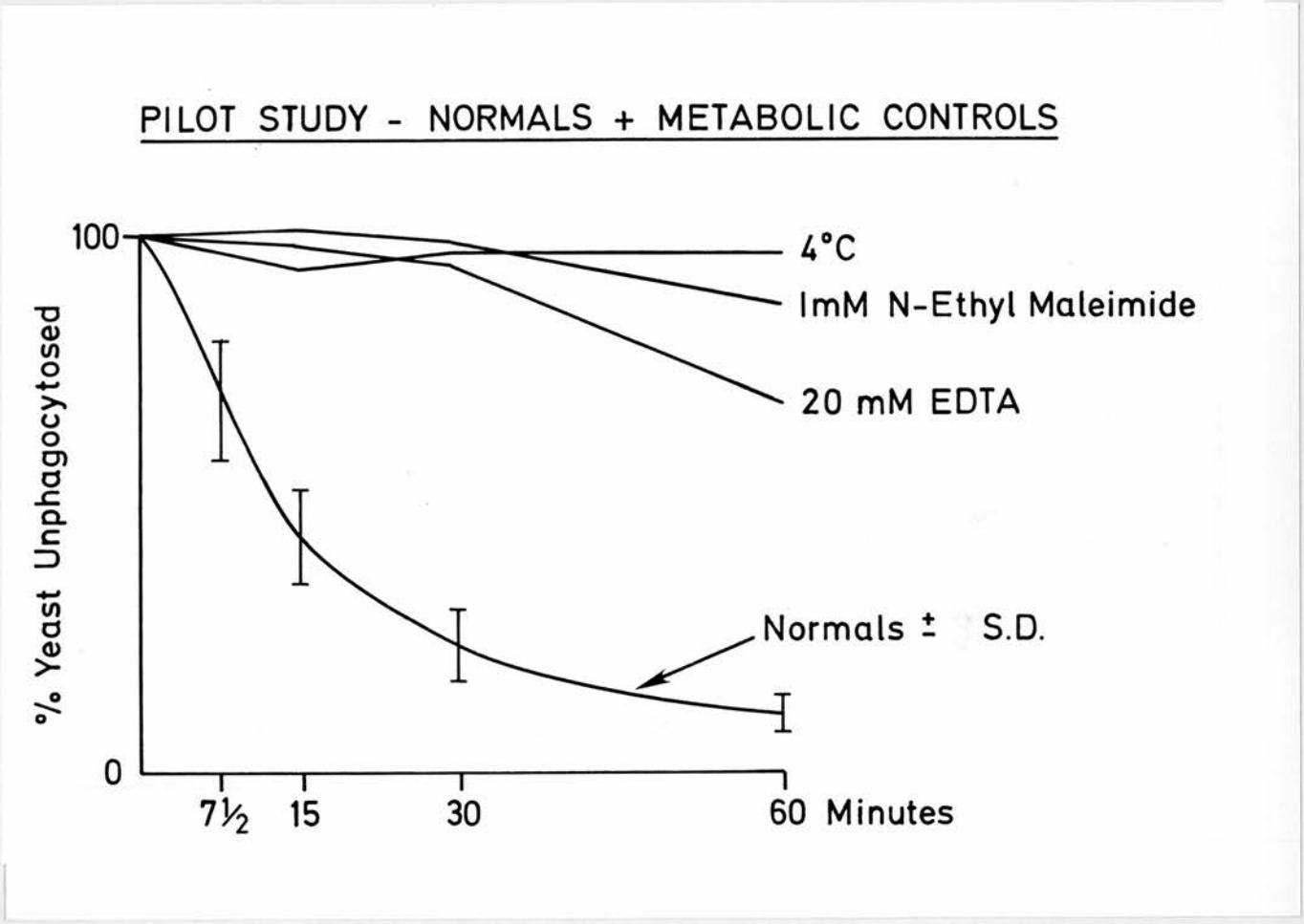
The time course of yeast uptake was next measured in monocytes from 20 normal controls using the "indirect" method and, to confirm that disappearance of yeast reflected phagocytosis rather than adherence, the effect of temperature and N-ethyl maleimide on yeast uptake were studied.

Using monocytes from the normal controls, yeast uptake was rapid and reproducible (Fig III.7). The mean (+/- s.d.) monocyte concentration was $5.4(+/-0.72)x10^6$/ml and mean yeast concentration $5.3(+/-0.86)x10^6$/ml.

Ten minutes preincubation of mononuclear cells in 1mM N-ethyl maleimide resulted in 89% inhibition of yeast uptake at 15 and 30 minutes of incubation (Fig III.7), confirming that disappearance of yeast depended on anaerobic glycolysis. EDTA also caused marked inhibition of phagocytosis demonstrating a requirement for divalent cations.

Similarly when the experiment was conducted at $4^{\circ}C$, 90% inhibition of yeast phagocytosis occurred (Fig III.7), confirming the requirement for metabolic processes.

Fig III.7 Time course of phagocytosis by monocytes from 20 normal controls and effect of temperature, N-ethyl maleimide and EDTA.



RESULTS OF ELECTRON MICROSCOPY ON PHAGOCYTOSING MONOCYTES.

To obtain qualitative confirmation that yeast phagocytosis was occurring, electron microscopic studies were carried out on monocytes after short incubations with yeast.

Electron microscopy of monocytes after 5 and 10 minutes incubation with yeast showed that ingestion was rapid and numerous monocytes containing yeast were seen. Typical electron micrographs showing phagocytic vacuoles are shown in Fig III.8a and 8b. At 5 minutes (Fig III.8a) , numerous ingested yeast were seen, while after ten minutes, the phagocytic vacuoles had become swollen and yeast disintegration was apparent (Fig III.8b).

Fig III.8a Electron microscopy of monocytes phagocytosing yeast.

5 minutes after addition of yeast to monocytes numerous phagocytosed yeast are seen and other yeast are bound by pseudopodia. A lymphocyte and erythrocyte are also present. (Mag X 8,000)

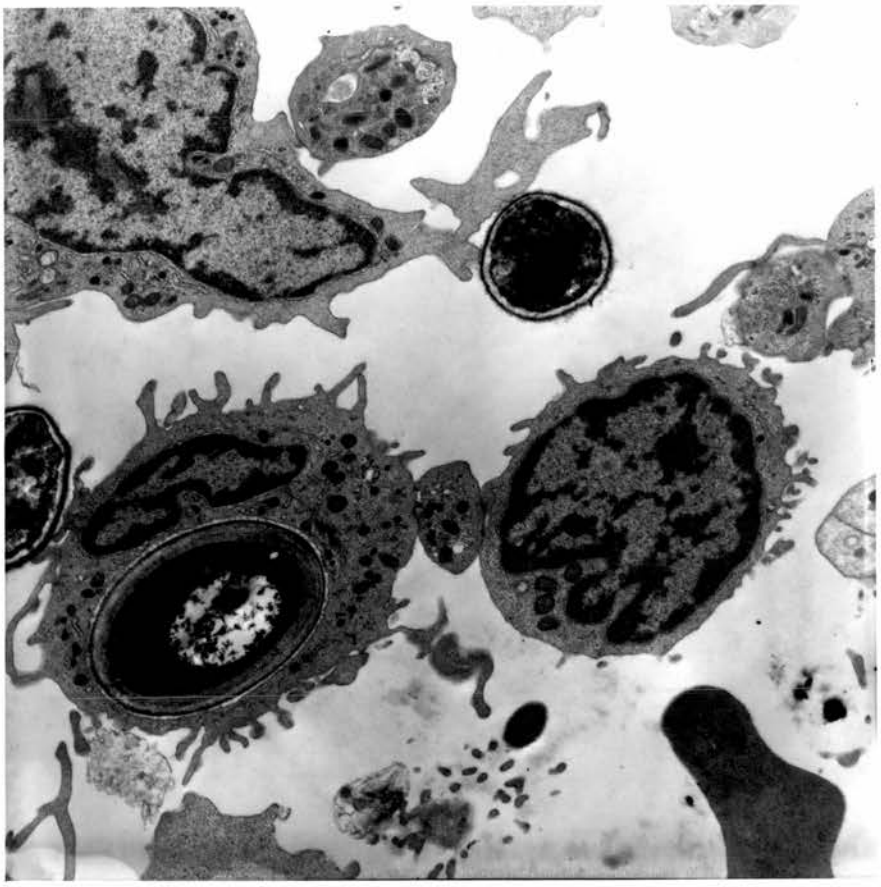
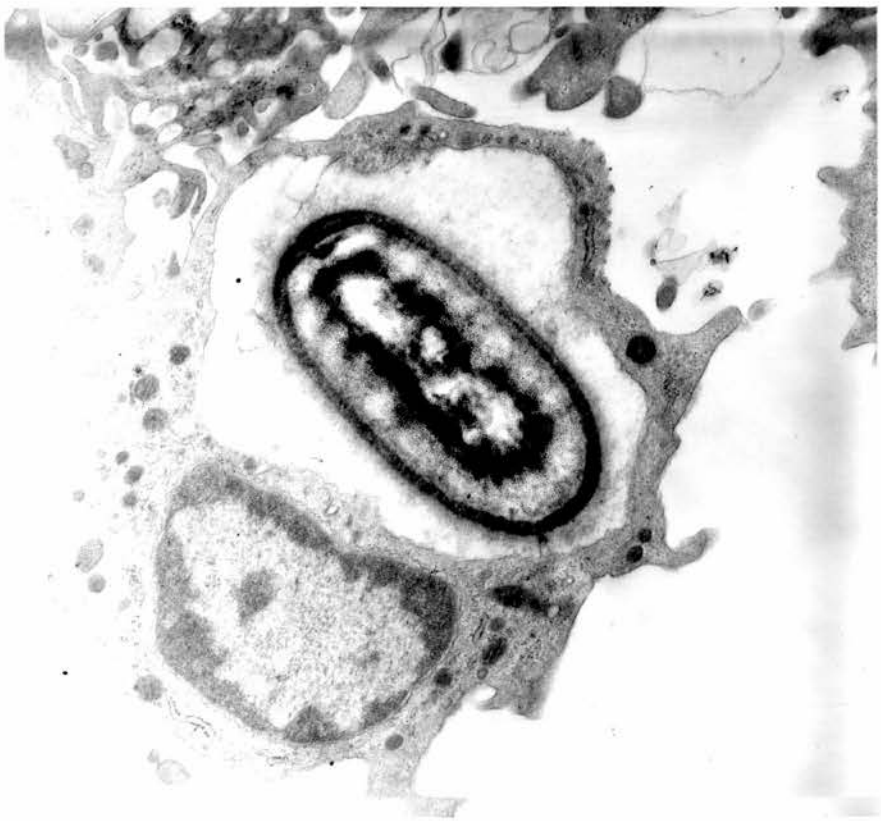


Fig III.8b Electron microscopy of monocytes phagocytosing yeast.

10 minutes after addition of yeast to monocytes, the phagocytic vacuoles are swollen and digestion of yeast is apparent.
(Mag X 12,000)



4.0 KINETICS OF YEAST PHAGOCYTOSIS.

INTRODUCTION.

In the following experiments the time course of phagocytosis by monocytes from a group of patients with rheumatoid arthritis was compared with that obtained from normal controls, and the kinetics of the "indirect" assay system evaluated with respect to yeast and monocyte concentration.

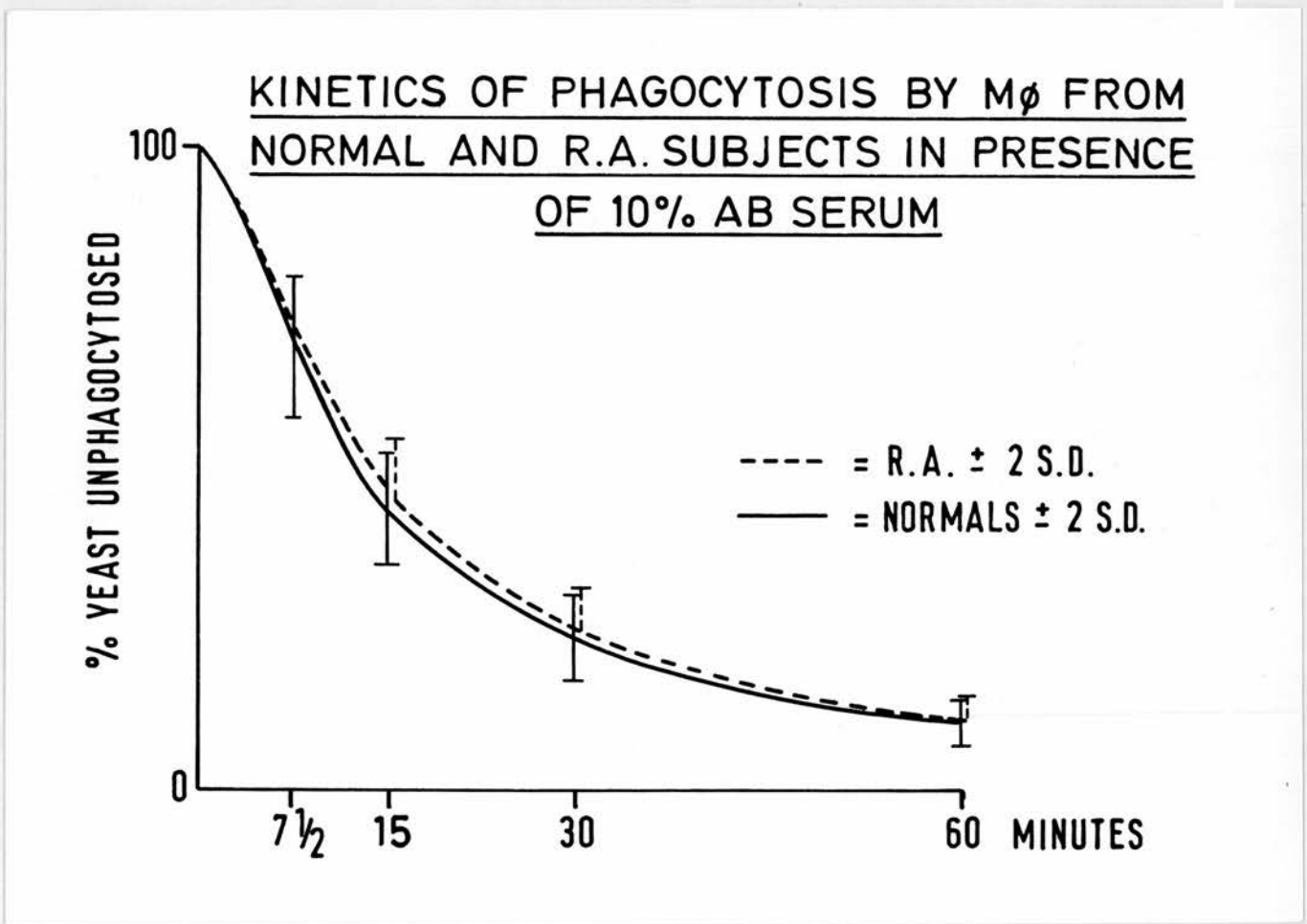
TIME COURSE OF PHAGOCYTOSIS BY MONOCYTES FROM NORMAL CONTROL SUBJECTS AND PATIENTS WITH RHEUMATOID ARTHRITIS.

The time course of yeast phagocytosis was similar in the 20 normal controls described above and 12 randomly selected patients with uncomplicated RA (Fig III.9). The mean (+/- S.D.) monocyte (Mo) and initial yeast (Y) concentrations were:

	Mo/ml	Y/ml
Controls	$5.40(+/-0.72)x10^6$	$5.30(+/-0.86)x10^6$
RA patients	$5.98(+/-0.64)x10^6$	$5.24(+/-0.63)x10^6$

Fig III.9 Time course of phagocytosis of C. albicans by monocytes from normal controls (n=20) and RA patients (n=12) in the presence of 10% AB serum:

The time course is identical in controls and RA patients.



KINETICS OF PHAGOCYTOSIS WITH RESPECT TO YEAST CONCENTRATION.

The disappearance of yeast from suspension appeared to be exponential, suggesting that yeast uptake followed first order kinetics:

$$\text{Rate of yeast uptake} = dY/dt = k \times Y,$$

$$\text{i.e.} \quad \ln(Y) = k \times t,$$

where Y = yeast concentration, t = time & k = a constant.
To test this hypothesis the time course data from the normal controls was plotted as ln(Y) versus time (Fig III.10). A straight line relationship was obtained over the first 30 minutes of incubation confirming that over this time period the kinetics are first order with respect to yeast concentration.

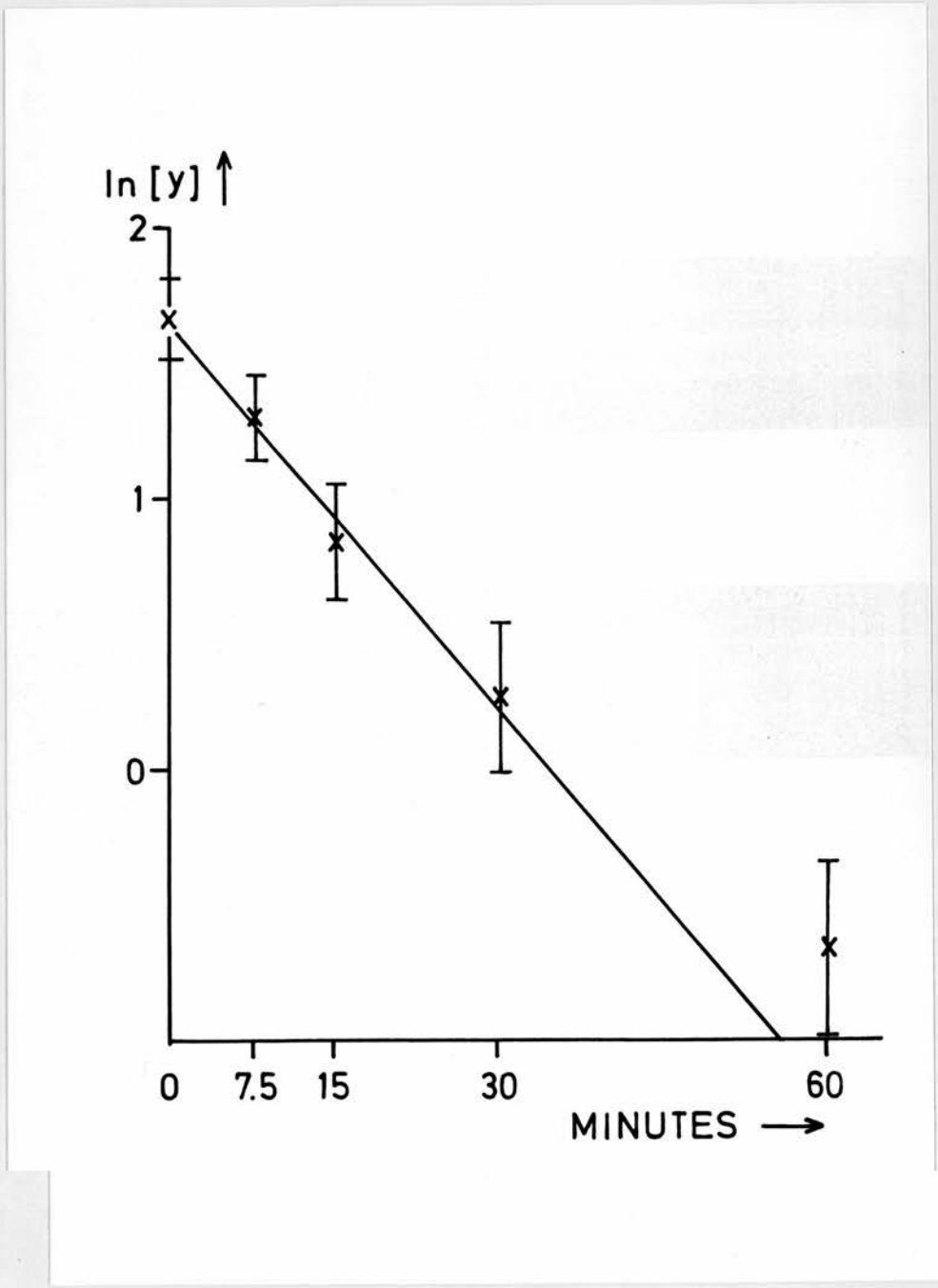
Thus, the rate of phagocytosis for a given monocyte concentration is given by:

$$\text{Rate of phagocytosis} = dY/dT = \ln(Yo/Yt)/t$$

where Yo and Yt are the yeast concentrations at time = 0 and t minutes respectively.

Fig III.10. Kinetics of phagocytosis by monocytes with respect to yeast concentration. (Each point represents the mean +/- 1 S.D. of 20 experiments)

Disappearance of yeast from suspension is exponential i.e. yeast uptake follows 1st order kinetics.



KINETICS OF PHAGOCYTOSIS WITH RESPECT TO MONOCYTE CONCENTRATION.

To determine whether the rate of phagocytosis was proportional to the monocyte concentration, the mean rate was measured over 30 minutes at three different monocyte concentrations: $1.0x10^6$/ml (n=9), $2.5x10^6$/ml (n=5) and $5.0x10^6$/ml (n=5). The monocytes were obtained from a single donor (NPH).

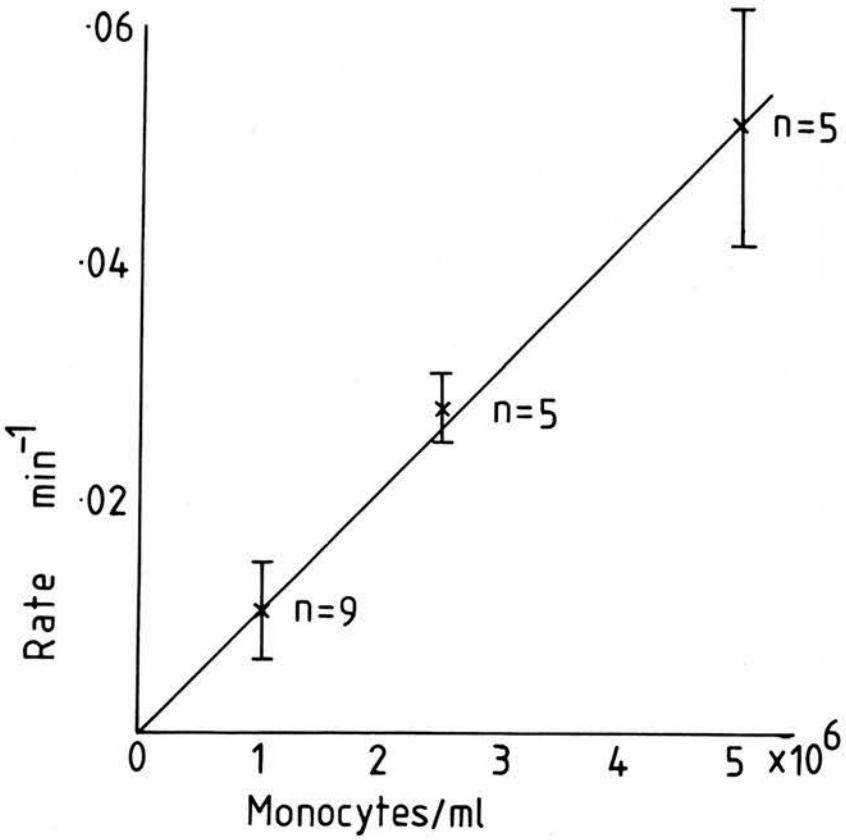
Measurement of the rate of phagocytosis in the presence of these three different monocyte concentrations confirmed that the rate of phagocytosis is proportional to the monocyte concentration (Fig III.11):

i.e.Rate of phagocytosis = $(\ln(Yo/Yt))/t = K \times Mo$,

where Mo = monocyte concentration and K is a constant.

Fig III.11. Kinetics of phagocytosis with respect to monocyte concentration:

The initial rate of phagocytosis of yeast is proportional to the monocyte concentration. Each point represents the mean +/- SD.



5.0 PHAGOCYTIC RATE CONSTANT.

Since yeast uptake follows first order kinetics and the rate of phagocytosis is proportional to the monocyte concentration, a value for a phagocytic rate constant "K" can be obtained from the expression:

$$K = (\ln(Yo/Yt))/(t \times Mo) \quad \text{mls/min/monocyte}$$

K provides a measure of the efficiency of phagocytosis and is analogous to the rate constant for a chemical reaction with two first order reactants.

The results of the above studies indicated that measurement of yeast uptake over a single 20 minute time interval would provide a value for the phagocytic rate constant "K". This would provide a measure of the efficiency of phagocytosis and obviate the need for a lengthy time course. However to make the method suitable for clinical use a number of modifications were made to the basic methodology.

6.0 MODIFICATIONS TO EXPERIMENTAL METHOD FOR MEASUREMENT OF PHAGOCYTIC RATE CONSTANT USING "INDIRECT" METHOD.

In order to reduce the volume of blood required for clinical studies the mean final concentration of cells used in the phagocytic assay was reduced from 5.0 to 1.5×10^6 monocytes/ml. To attain this final concentration only 10mls of venous blood were required.

To reduce possible interexperimental variation in degree of opsonisation of phagocytic particles and to avoid exposure of monocytes to complement activation products, all yeast were

preopsonised in a single batch and stored in aliquots in liquid nitrogen until ready for use. Experiments were also performed to investigate the type of opsonins present on the preopsonised yeast particles (vide infra).

To minimise errors due to pipetting, aliquots of cells and yeast were placed in all six wells of the Teflon block in each experiment, rather than only one well.

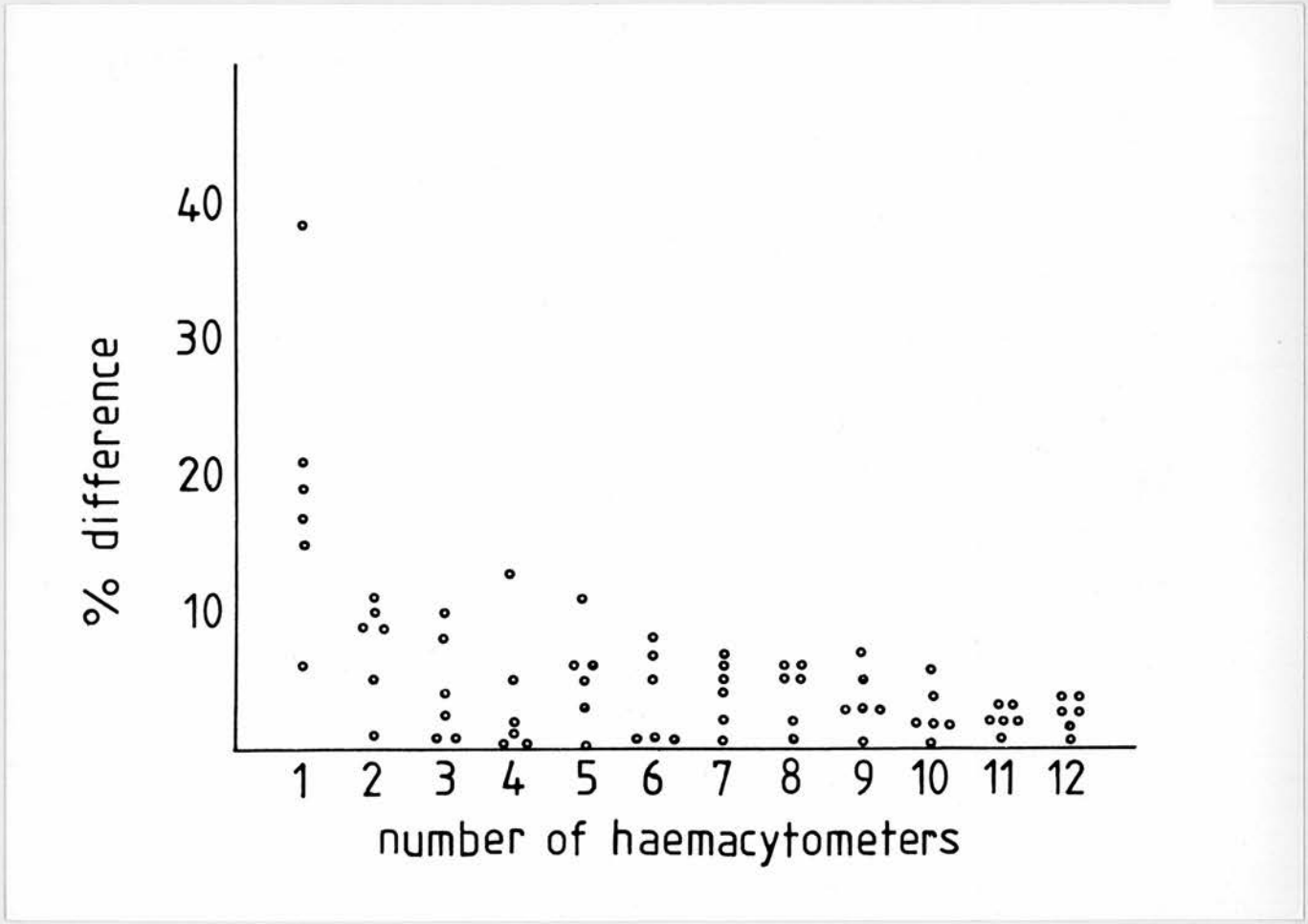
The method for monitoring the fall in extra-cellular yeast concentration involves the use of haemacytometers and it was noted that there was frequently wide variation in counts between individual haemacytometers. Since this was a potential source of error further experiments were performed to determine the minimum number of haemacytometer counts which should be performed to obtain a reproducible estimate of yeast concentration at each time point and thereby minimise this problem.

The full details of the method used to investigate this problem are described above (p194). Repeated haemacytometer counts showed that to achieve less than 5% difference in the mean count between opposite sides of the haemacytometers, a minimum of 11 or 12 haemacytometer counts should be performed (Figure III.12).

In all subsequent experiments therefore, 12 haemacytometers were used to monitor the change in yeast concentration. The complete method incorporating these modifications for measuring the phagocytic rate constant is described above (pp196-199).

Fig III.12 Haemacytometer errors:

The %age difference between the mean count of yeast in opposite sides of haemacytometers diminishes with the use of increasing numbers of haemacytometers. Approximately 12 haemacytometer counts are required to minimise this source of error. (n=6)



7.0 CONTROL STUDIES ON OPSONISED PARTICLES - THE NATURE OF THE OPSONINS.

OPSONINS PRESENT ON C. ALBICANS OPSONISED WITH SERUM

In some studies C. albicans preopsonised with human AB serum was used. Direct immunofluorescence showed that this particle was coated both with immunoglobulins (IgA, G & M) and complement C3/C3c (β1c/β1a). The rate constant measured with this yeast is designated Ks.

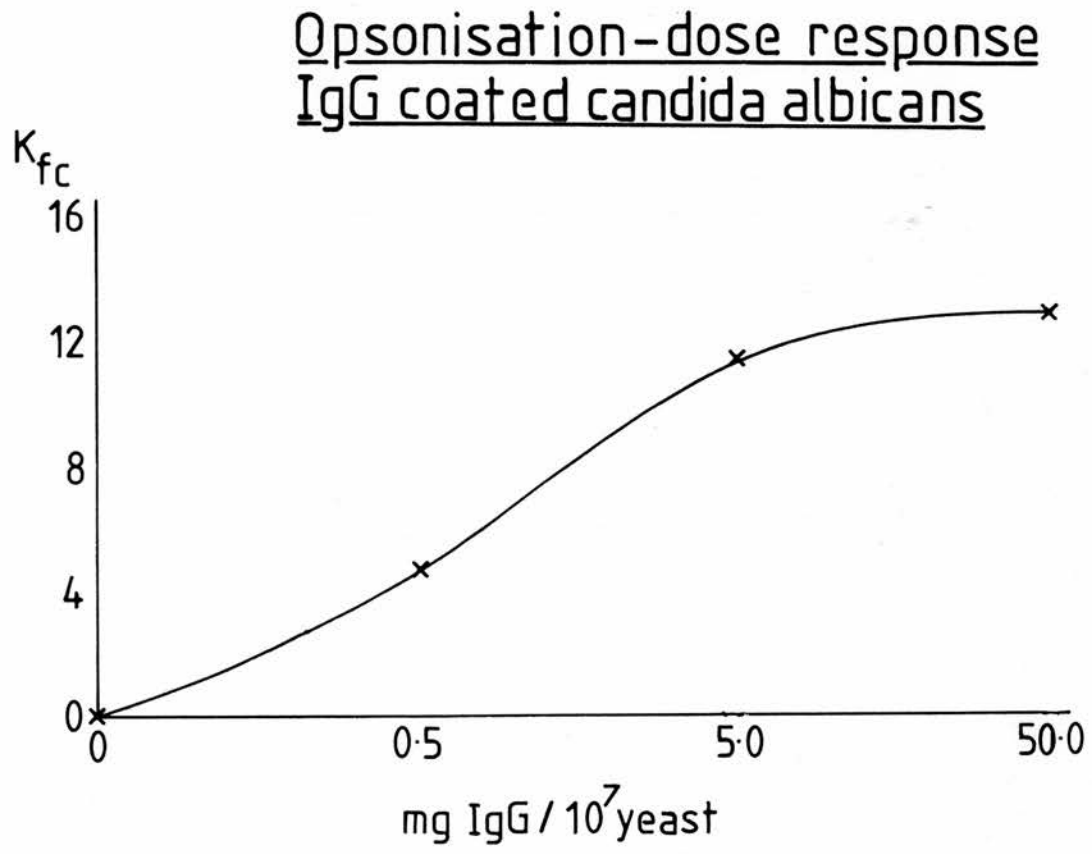
OPSONISATION DOSE RESPONSE STUDIES AND OPSONINS PRESENT ON C. ALBICANS OPSONISED WITH POOLED HUMAN IgG

In later studies C. albicans preopsonised with pooled human IgG was used to measure Fc receptor mediated uptake. Opsonisation dose response curves were preformed to establish the optimum ratio of yeast to IgG (Fig III.13). The standard opsonisation ratio chosen for all subsequent experiments was 50mg IgG/10^8 yeast in 2mls of PBS.

Treatment of these yeast with goat F(ab')2 anti-Fc resulted in 95% inhibition of phagocytosis, confirming that uptake is Fc receptor dependent. Treatment of yeast with nonimmune goat F(ab')2 had no effect.

FIG III.13. Opsonisation dose response curve for C. albicans opsonised with pooled human IgG.

Ratio of yeast to opsonin is plotted against rate of phagocytosis (Kfc 10^{-4} mls/min/monocyte).



OPSONISATION DOSE RESPONSE STUDIES AND OPSONINS PRESENT ON S. CEREVISIAE OPSONISED WITH SERUM

To obtain a measure of rates of "complement (C3) receptor" mediated ingestion (Kc), S. cerevisiae preopsonised in fresh human serum was used (Rivero et al 1979), and dose response curves performed to establish the optimum ratio of yeast to serum (Fig III.14). A ratio of 0.4mls.serum/10^7 yeast was used in subsequent experiments.

Preliminary immunofluorescence studies showed that the particles were heavily coated with C3/C3c (β1c/β1a) and no immunoglobulin was detected. Furthermore, after heat inactivation of the serum, opsonisation of the yeast was abolished (Fig III.14).

To determine more precisely which complement components were mediating ingestion, and to exclude the presence of small amounts of IgG, the serum-opsonised yeast particles were incubated with F(ab')2 fragments of goat IgG directed against various complement components before offering the yeast for phagocytosis in the assay.

F(ab')2 anti-C1q caused 15% inhibition and F(ab')2 anti-C3 caused 85% inhibition at the highest dilutions tested. F(ab')2 fragments of IgG anti-C4, anti-β1H, anti-Fc and nonimmune IgG had no effect. Furthermore, simultaneous treatment with anti-C1q and anti-C3 caused 100% inhibition (Table 6.2). Nonimmune goat F(ab')2 had no effect.

Fig III.14. Opsonisation dose response curve for S. cerevisiae opsonised with human serum.

Ratio of yeast to opsonin is plotted against rate of phagocytosis (Kc 10^{-9} mls/min/monocyte). Yeast opsonised with heat inactivated serum are not significantly phagocytosed.

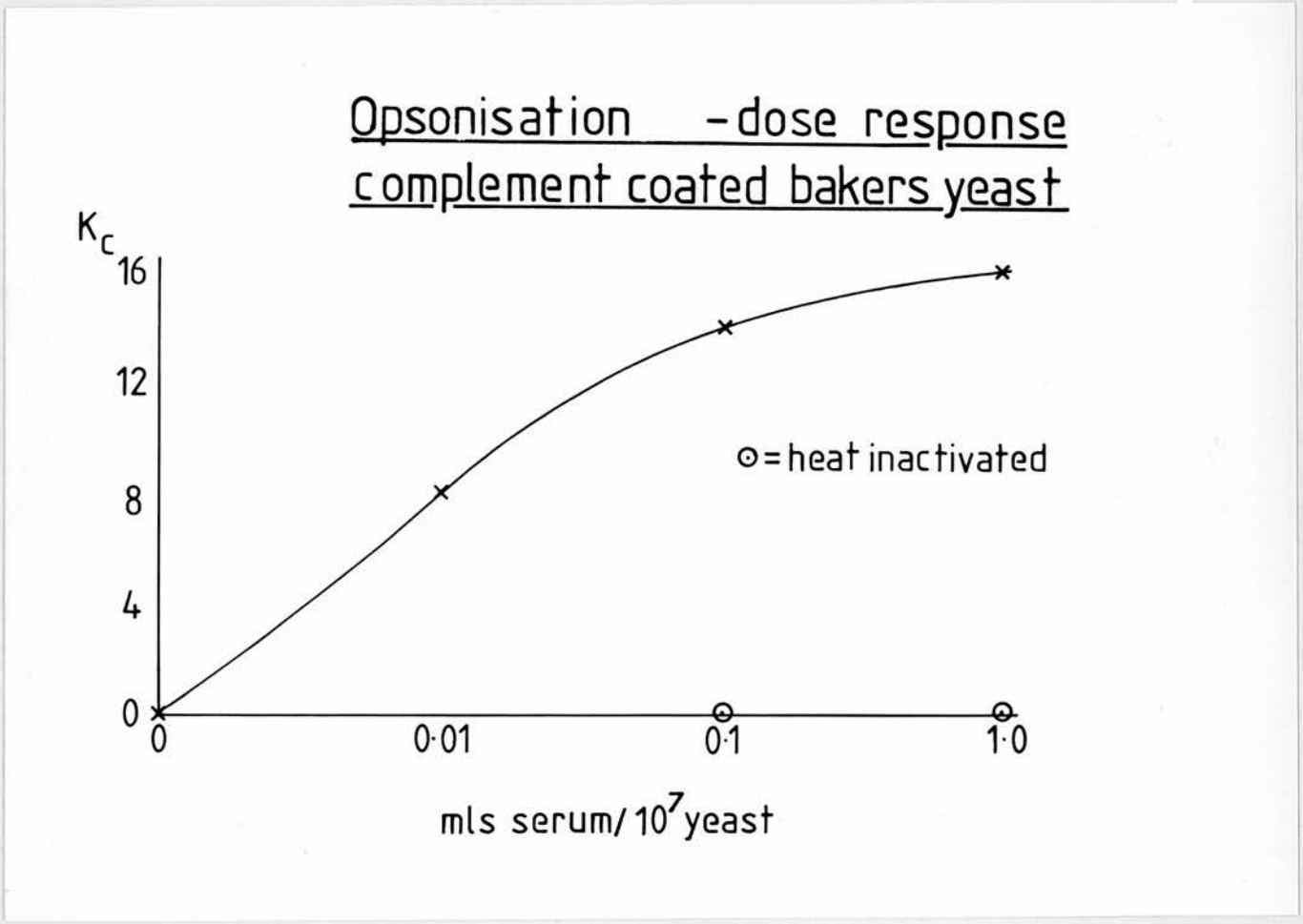


Table 6.2 Effect on phagocytosis of treating "complement" coated S. cerevisiae with F(ab')2 anti-C1q, C3, C4, β1H or anti-IgG/Fc. Result expressed as %age inhibition of phagocytosis:

Dilution	C1q	C3	C4	β1H	Fc	C1q+C3
1/1	15	87	0	0	0	100
1/5	15	80	0	0	0	ND
1/10	15	72	0	0	0	ND

CONFIRMATION THAT RATE OF PHAGOCYTOSIS IS PROPORTIONAL TO MONOCYTE CONCENTRATION

To ensure that the kinetics of phagocytosis were unchanged using yeast preopsonised with serum or IgG or complement alone, the rate of phagocytosis was measured over 20 minutes in the presence of concentrations of monocytes ranging from 1.0 to 2.0×10^6/ml obtained from healthy control subjects. The experiments were repeated for C. albicans opsonised in serum, S. cerevisiae opsonised in serum and C. albicans opsonised in IgG.

In each case the rate of phagocytosis was proportional to the monocyte concentration (Figs III.15,16,17).

Fig III.15 Rate of phagocytosis of C. albicans preopsonised with serum is proportional to monocyte concentration.

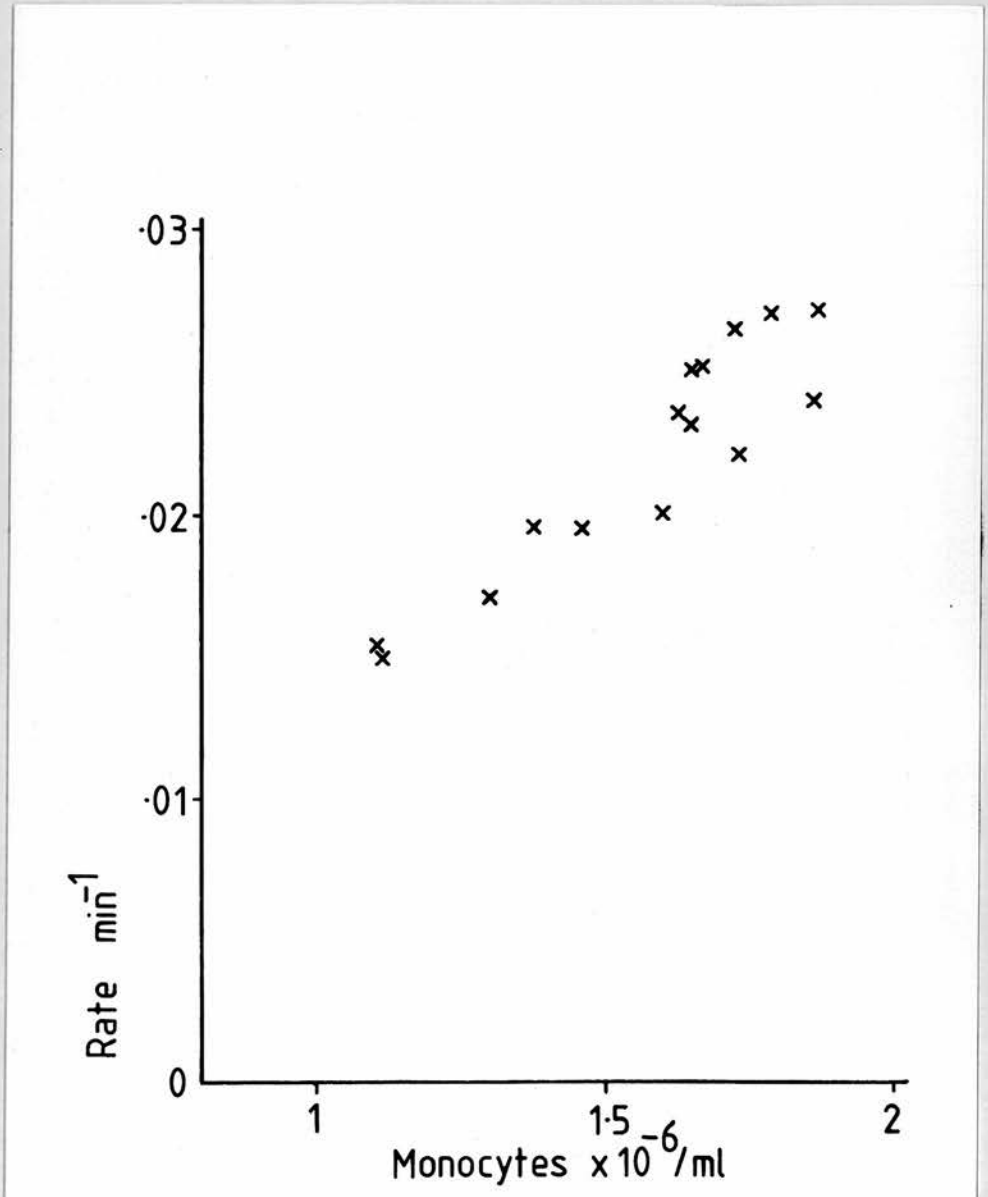


Fig III.16 Rate of phagocytosis of C. albicans preopsonised with IgG is proportional to monocyte concentration.

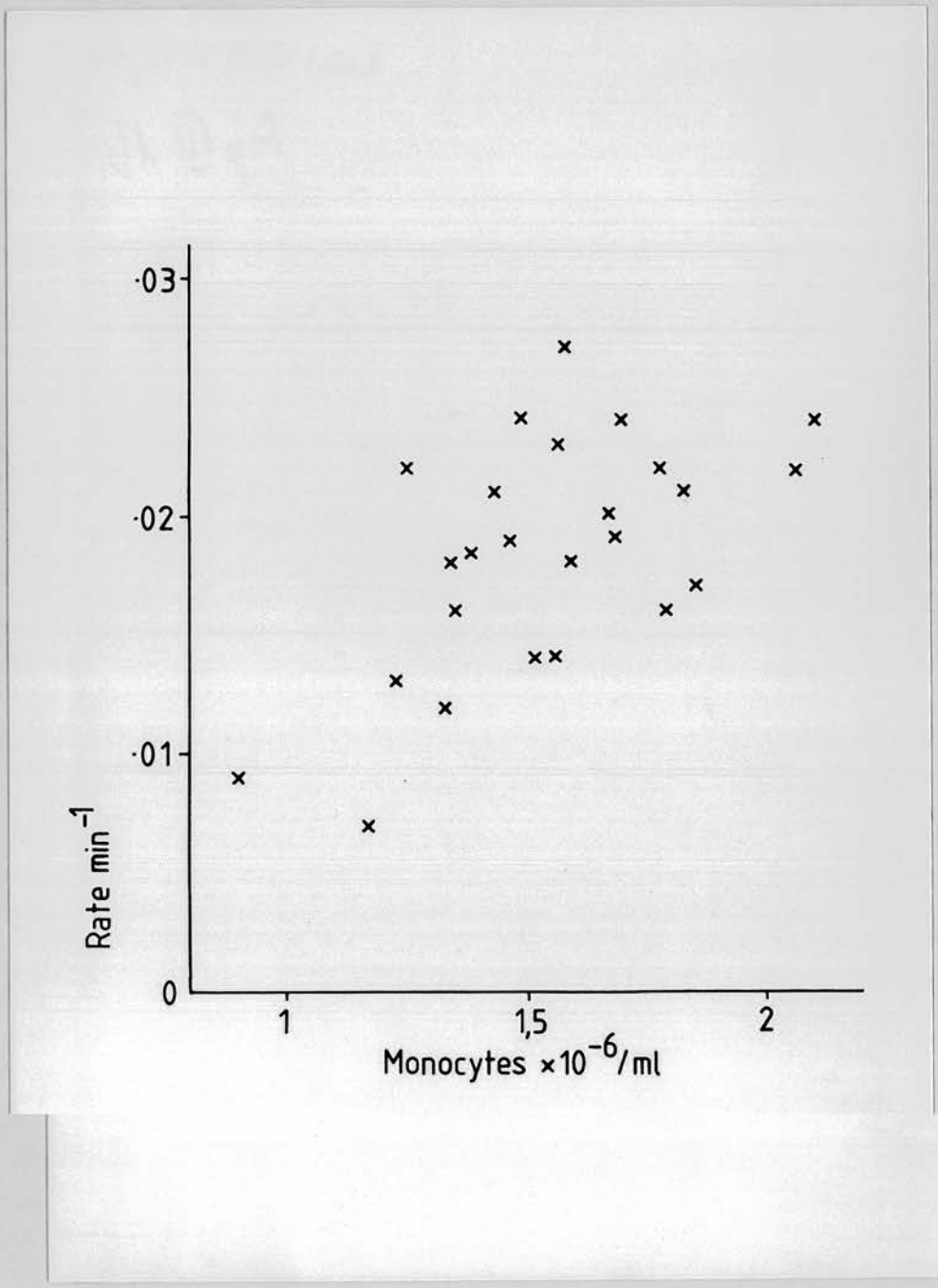
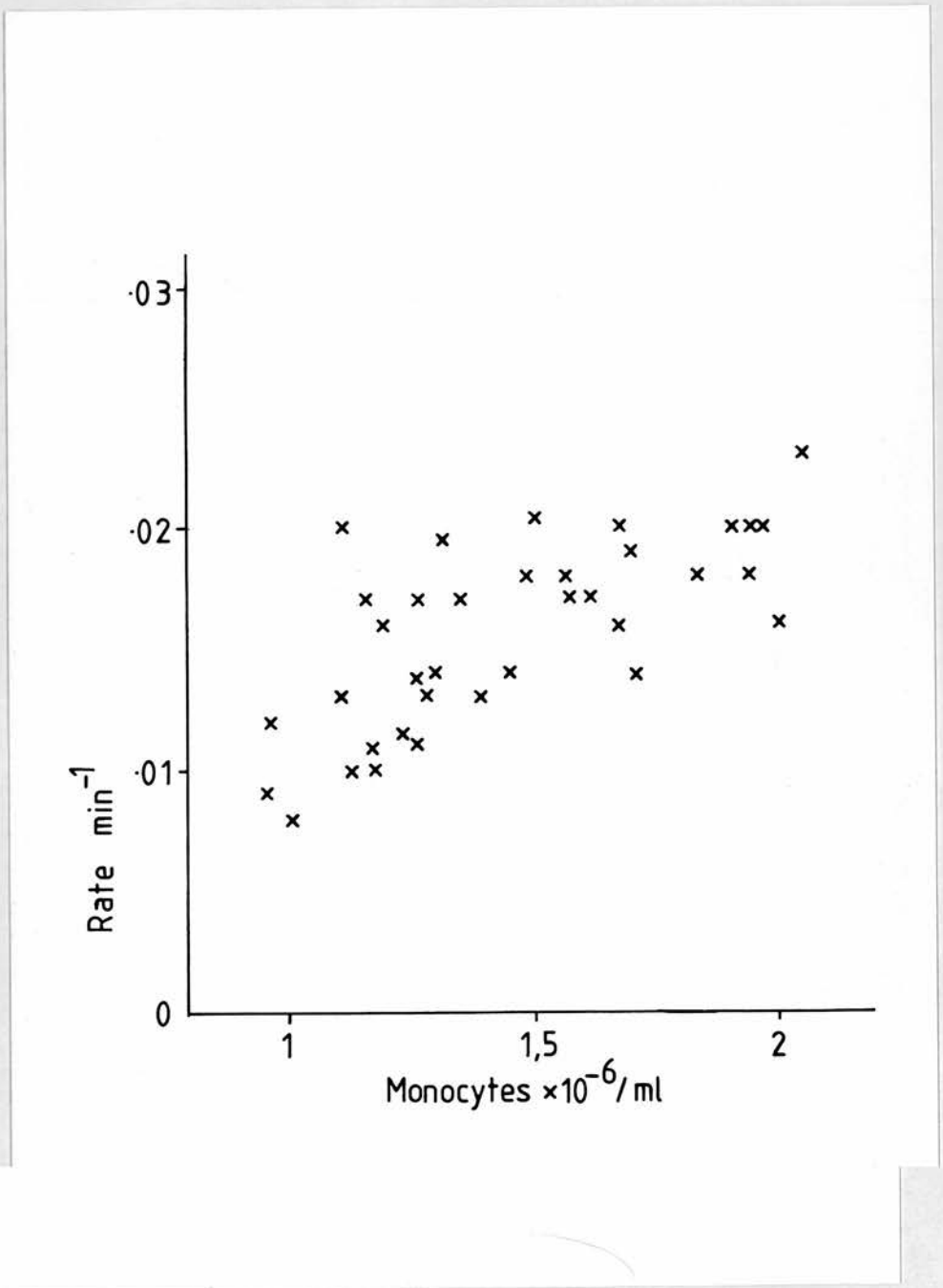


Fig III.17 Rate of phagocytosis of S. cerevisiae preopsonised with serum (complement) is proportional to monocyte concentration.



6.0 RESULTS OF METABOLIC CONTROL STUDIES.

INTRODUCTION.

To confirm that apparent uptake of complement coated S. cerevisiae and IgG coated C. albicans was dependent on phagocytosis rather than adherence the effect of various metabolic inhibitors on the rate constants Kc and Kfc was investigated.

N-ETHYL MALEIMIDE.

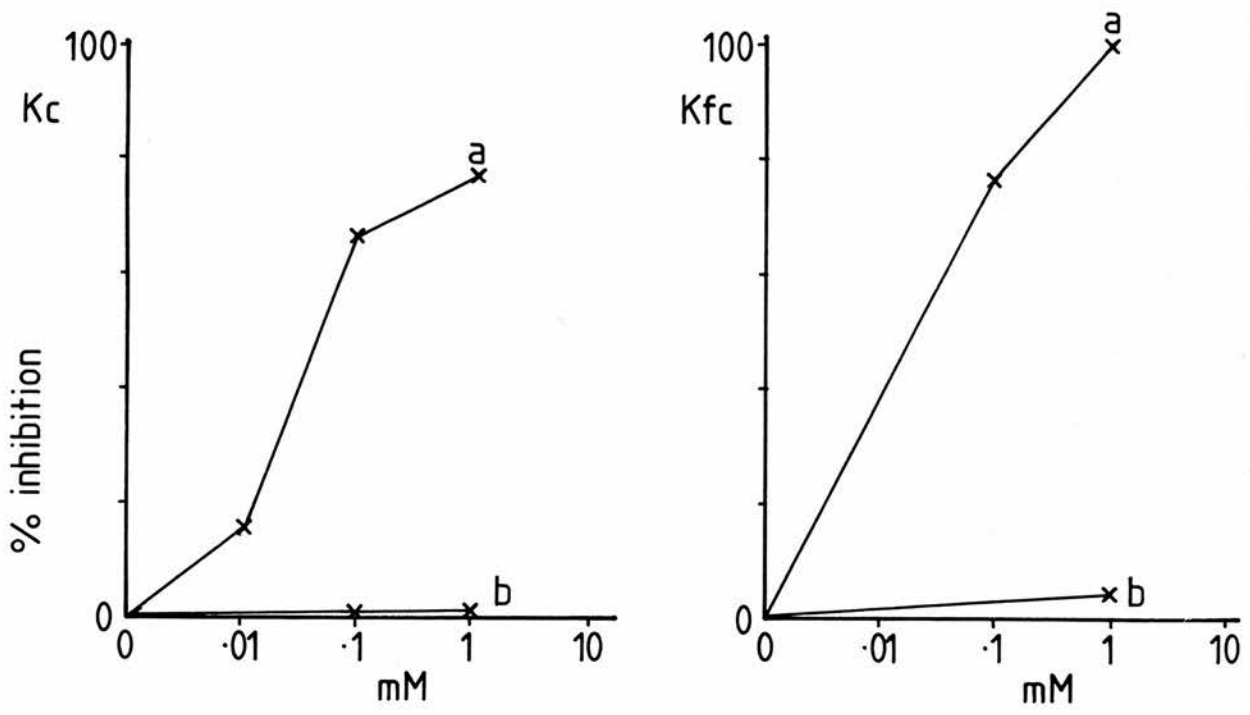
Ten minute preincubation of mononuclear cells with 0.01, 0.1 and 1.0mM N-ethyl maleimide, caused dose dependent reduction of both Kc and Kfc suggesting that uptake of both types of yeast particle was dependent on anaerobic glycolysis and that adherence made only a small contribution to disappearance of yeast (Fig III.18).

SODIUM CYANIDE

Preincubation of cells for 60 minutes with up to 1mM cyanide had no effect on either Kc or Kfc confirming that aerobic mitochondrial metabolism does not normally provide significant amounts of energy for phagocytosis (Fig III.18).

Fig III.18 Effect of N-ethyl maleimide and sodium cyanide on rates of phagocytosis of either IgG (Kfc) or "complement" (Kc) coated yeast.

(a) N-ethyl maleimide causes dose dependent inhibition of phagocytosis of either particle while (b) sodium cyanide has no effect.



2-DEOXY-GLUCOSE

Preincubation of cells with 10mM 2-deoxy glucose for either 20 or 60 minutes produced inhibition of both Kc and Kfc confirming dependence of yeast phagocytosis on glycolysis (Fig III.19). However, at most only 50% inhibition of phagocytosis occurred. Rephosphorylation of ADP to ATP by stores of creatine phosphate provides the cell with a major source of ATP during phagocytosis; in the resting cell ATP is utilised for phosphorylation of creatine to creatine phosphate which provides a high energy phosphate store. Thus in order to deplete the cell of all sources of ATP which could be utilised for phagocytosis, preincubation was performed in the presence of 2-deoxy glucose and cyanide simultaneously.

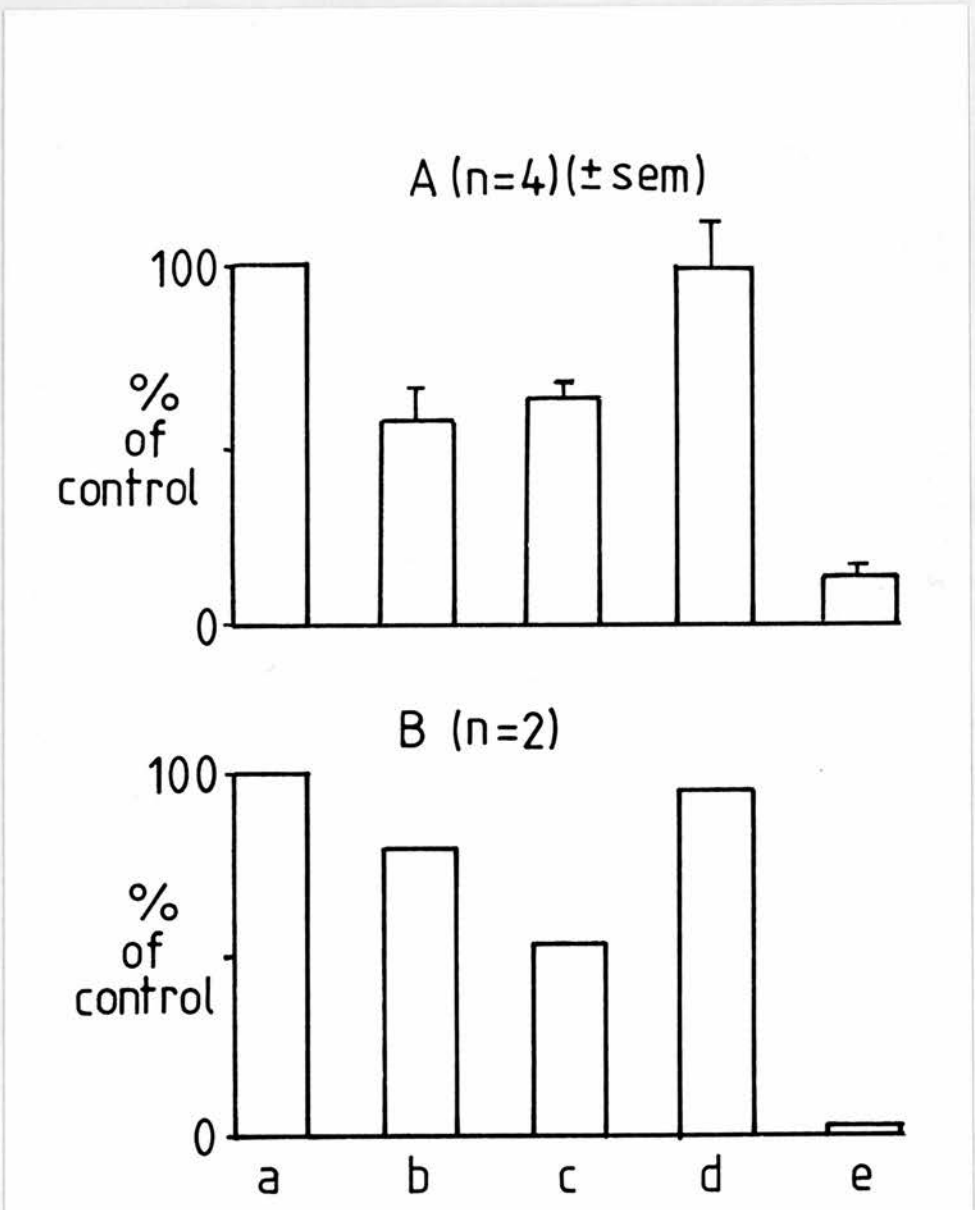
2-DEOXY-GLUCOSE + SODIUM CYANIDE

Preincubation of cells for 60 minutes with 10mM 2-deoxy glucose and 1mM sodium cyanide produced 98% inhibition of both Kc and Kfc (Fig III.19), confirming that uptake of yeast is dependent on active metabolic processes.

Fig III.19 Effect of 2-deoxy glucose (2DG), or 2DG + cyanide on phagocytosis of A)"complement" coated yeast and B)IgG coated yeast.

Preincubation with 10mM 2DG produced approximately 50% inhibition of phagocytosis of either IgG or "complement" coated yeast, cyanide had no effect, but preincubation with 10mM 2DG + 1mM cyanide caused virtually complete inhibition of phagocytosis.

a) = Control cells preincubated with medium (20 or 60 min)
b) = 20 minute preincubation with 10mM 2DG
c) = 60 minute " " " "
d) = 60 minute " " 1mM cyanide
e) = 60 minute " " 10mM 2DG + 1mM cyanide



REQUIREMENT FOR DIVALENT CATIONS AND EFFECT OF EDTA.

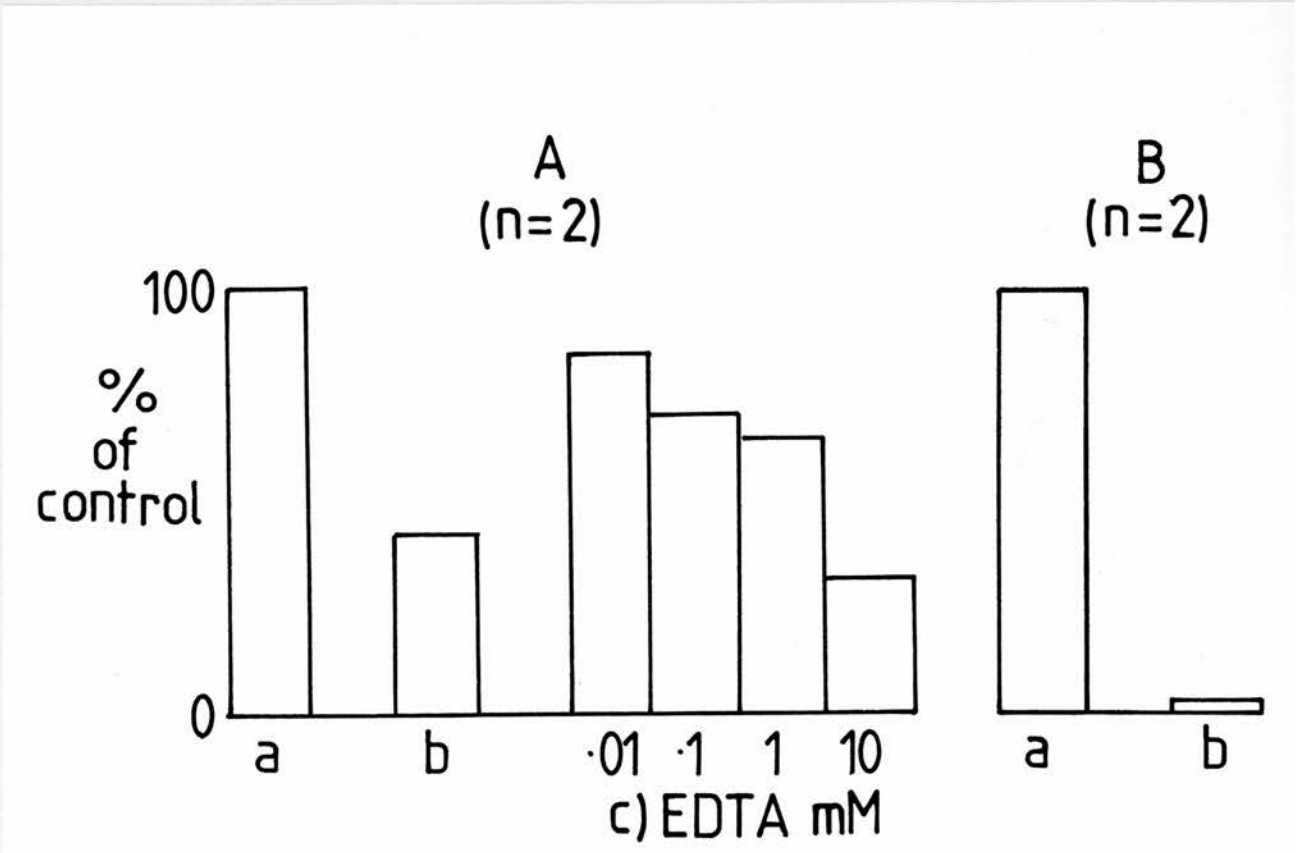
Phagocytosis of IgG coated yeast was completely inhibited in the absence of Ca++ and Mg++. Uptake of "complement" coated yeast was only partially inhibited by omission of divalent cations and was inhibited to a similar degree by preincubation of monocytes in HBSS with 10mM EDTA for 10 minutes (Fig III.20).

Fig III.20. Effect of Ca++ and Mg++ free medium on phagocytosis of A)"complement" coated yeast and B)IgG coated yeast, and effect of EDTA on phagocytosis of "complement" coated yeast.

a) = Control cells preincubated 10 minutes with medium

b) = Cells preincubated 10 minutes with Ca++/Mg++ free medium

c) = Cells preincubated 10 minutes with EDTA (complement coated yeast only).



SECTION IV: RESULTS OF STUDIES ON PATIENTS WITH RHEUMATOID ARTHRITIS.

1.0 RATES OF PHAGOCYTOSIS OF C. ALBICANS PREOPSONISED WITH AB SERUM, BY MONOCYTES FROM NORMAL CONTROLS AND PATIENTS WITH RHEUMATOID ARTHRITIS.

In the initial clinical study, the rate constant Ks for combined IgG and "complement" mediated monocyte phagocytosis was measured using C. albicans preopsonised with serum.

SUBJECTS

Controls: Fourteen healthy hospital employees (10 female, 4 male), mean age 35 years (range 20-63).

Patients: Fourteen patients with classical or definite rheumatoid arthritis (RA) (Ropes et al 1959) (9 female, 5 male), mean age 54 years (range 30-76). Five of the patients had cutaneous rheumatoid vasculitis (RV) (1 male, 4 female), mean age 59 years. One patient with vasculitis was restudied after resolution of the vasculitic episode.

Drug therapy: Twelve RA patients were receiving nonsteroidal anti-inflammatory drugs (NSAID) alone and three were also receiving corticosteroids. Of these three, one with vasculitis was receiving prednisolone 6mg/day, and two patients without vasculitis were receiving prednisolone 5mg/day and ACTH 20 units/day respectively.

The appearance of typical cutaneous vasculitic lesions is shown in Figs IV.1 and 2.

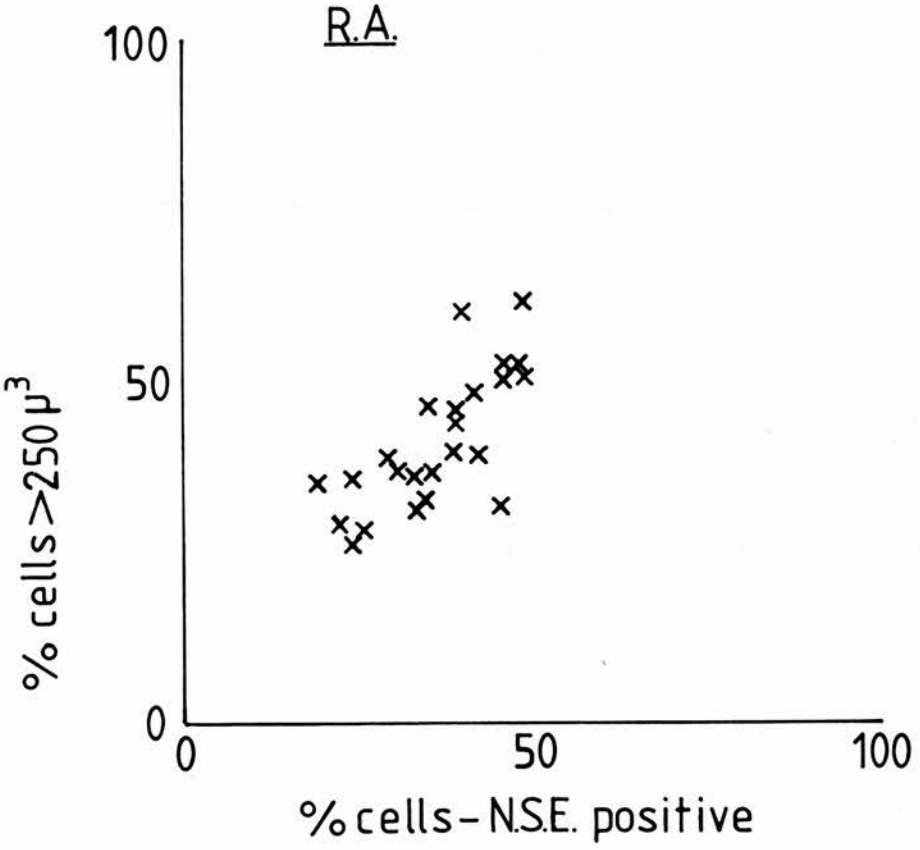
Figs IV.1 & 2 Appearance of typical rheumatoid cutaneous vasculitic lesions:

VALIDATION OF COULTER SIZING METHOD FOR PERFORMING MONOCYTE DIFFERENTIAL COUNTS ON CELLS FROM RA PATIENTS.

To confirm that the Coulter sizing technique for performing monocyte differential counts was valid for mononuclear cells obtained from RA patients, the method was compared with NSE staining using cells from 24 RA patients.

As in the normal controls, a good correlation was found between the percentage of monocytes in mixed mononuclear cell populations estimated by NSE staining and the percentage estimated by Coulter sizing ($n=24$, $r=0.71$, $p<0.001$),(Fig IV.3).

Fig IV.3 %ages of monocytes in mixed mononuclear populations from RA patients.
Differential counts obtained by NSE staining correlate well with the Coulter "sizing" method in which monocytes are defined as cells of volume greater than 250 cub microns (n=24;r=0.71;p<0.001):



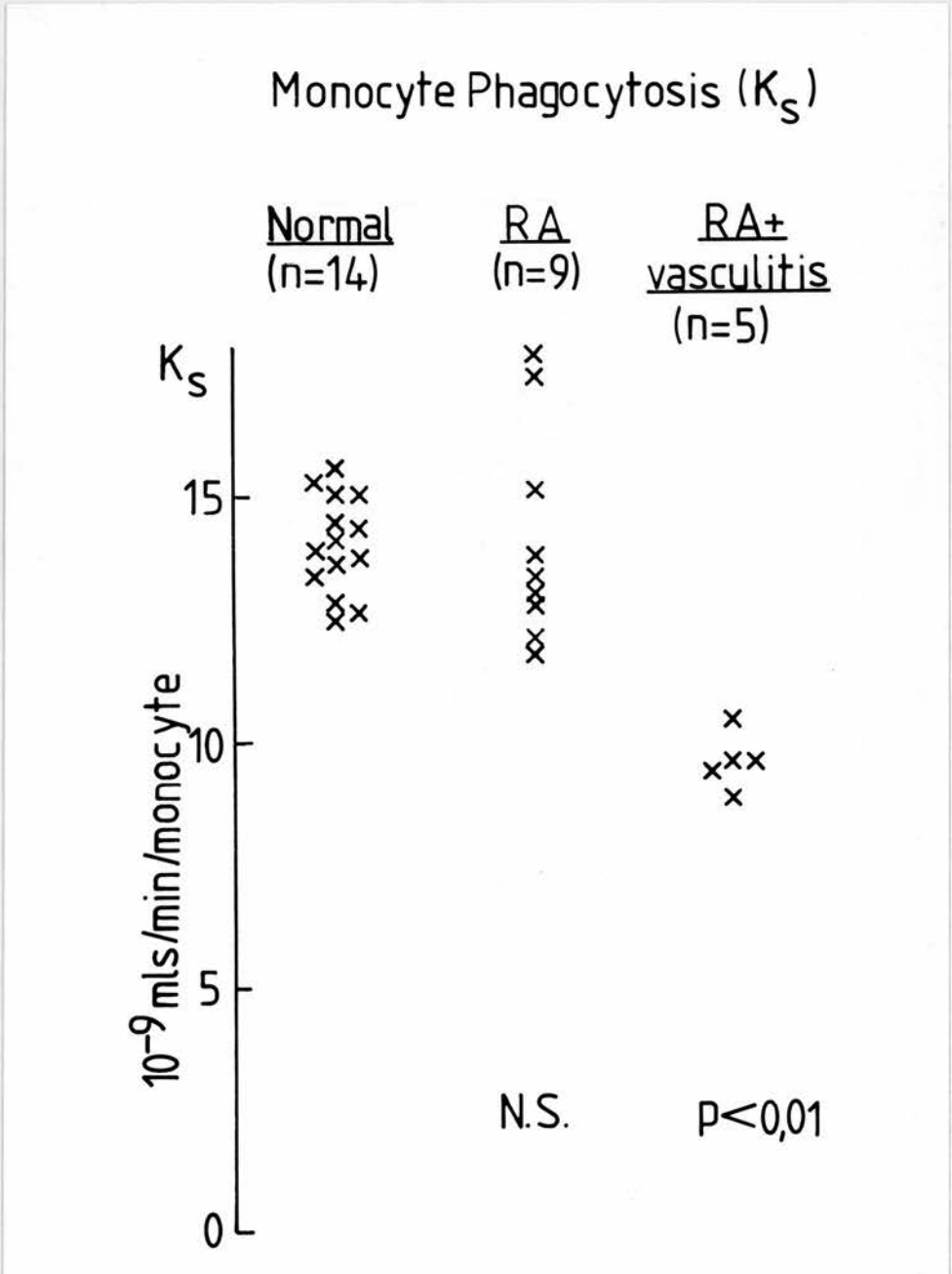
RATE CONSTANT FOR MONOCYTE PHAGOCYTOSIS OF C. ALBICANS PREOPSONISED WITH AB SERUM - STUDIES OF NORMAL SUBJECTS AND RA PATIENTS WITH AND WITHOUT VASCULITIS.

Mononuclear cells were separated from venous blood obtained from the above subjects and the concentration of monocytes determined using the Coulter counter. The monocyte phagocytic rate constant "Ks" for uptake of C. albicans opsonised with AB serum was then measured for each subject as described above (p196).

There was no difference in the rate constant Ks between normal controls and patients with uncomplicated RA, but Ks was significantly reduced in patients with active vasculitis ($p<0.01$, rank sum test) (Fig IV.4). In one patient who was restudied after resolution of the vasculitic episode Ks was found to have returned to normal.

Fig IV.4 Rate constant Ks for phagocytosis of serum opsonised C. albicans by monocytes from normal controls and RA patients with or without cutaneous vasculitis:

Rates of phagocytosis by monocytes from patients with vasculitis are significantly reduced compared to the other two groups ($p<0.01$; rank sum test).



SEROLOGICAL STUDIES

The results of serological studies are shown in Table 6.3. Although there is considerable overlap in the results the mean (+/- S.D.) serum haemolytic complement level is lower in the vasculitic subjects (42+/-32 %) than in the uncomplicated RA patients (75.4+/-29 %) (Not significant; Students t-test) suggesting the presence of circulating, complement activating immune complexes. All but one patient was seropositive for rheumatoid factor in either the SHCT or latex agglutination test.

Table 6.3 Results of serological studies in RA patients:

Patient	ESR	TITRE		CH50
	mm/1st hr.	SHCT	LATEX	ANF	%
RV1	42	512	+++	++	50
RV2	42	-ve	-ve	-ve	85
RV3	56	1024	+++	++	18
RV4	40	128	+++	+++	15
RV5	90	128	N.D.	+	N.D.
RA1	11	256	+++	+++	83
RA2	4	256	++	-ve	108
RA3	30	128	++	+	108
RA4	97	1024	N.D.	N.D.	21
RA5	80	N.D.	+++	++	54
RA6	71	128	+++	++	70
RA7	35	-ve	+++	++	51
RA8	54	128	+++	+	86
RA9	6	128	+++	-ve	98

RV = rheumatoid vasculitis

RA = rheumatoid arthritis without vasculitis

IMMUNOFLUORESCENCE STUDIES

In three experiments where reduced rates of phagocytosis had been found, indirect immunofluorescence was performed on the mononuclear cells using goat anti-human gamma globulin and anti-human complement (βlc/βla) in an attempt to identify monocyte associated immune complexes.

No evidence of cytoplasmic or membrane bound immune complexes was found.

DISCUSSION

Since the yeast used in this study were coated with both immunoglobulin and complement, it was not clear whether Fc receptors or complement receptors or both were responsible for the reduction in rates of phagocytosis of preopsonised yeast by monocytes from vasculitis patients. To examine these possibilities, further studies were performed using C. albicans preopsonised with IgG to measure Fc receptor mediated phagocytosis and S. cerevisiae preopsonised with serum to measure "complement" receptor mediated phagocytosis.

2.0 RATES OF PHAGOCYTOSIS OF YEAST PREOPSONISED WITH EITHER "COMPLEMENT" OR IMMUNOGLOBULIN (IgG) BY MONOCYTES FROM NORMAL CONTROLS AND PATIENTS WITH RHEUMATOID ARTHRITIS.

In the next group of subjects studied, the rate constants for "complement" and Fc receptor mediated phagocytosis, Kc and Kfc respectively, were measured separately. Kc was measured using S. cerevisiae preopsonised with serum and Kfc using C. albicans preopsonised with IgG as described above (p196).

SUBJECTS.

Controls: Twenty three healthy hospital employees (10 male, 13 female), mean age 32.2 years (range 21-64).

Patients: Twenty six patients with definite or classical RA (14 female, 12 male), mean age 59.4 years (range 35-73) were divided into four groups: Group A - active vasculitis (n=9); Group B - 3 patients from Group A whose vasculitis was inactive plus 2 patients with a history of vasculitis (n=5); Group C - multiple nodules (n=9); Group D - patients with miscellaneous conditions associated with RA (includes 1 patient from Group A who developed pyoderma gangrenosum); pyoderma gangrenosum (n=2), pericarditis (n=1), primary biliary cirrhosis (n=1), paravertebral abscess (n=1), Feltys syndrome (n=1), pyarthrosis, sacral ulceration and secondary Sjogrens syndrome (n=1). Five patients were studied serially to examine the relationship between abnormalities of phagocytosis, clinical status and serological parameters. Details of drug therapy are shown in Table 6.4.

Table 6.4 RA patients - Drug therapy:

	Patient Groups *			
DRUGS	A	B	C	D
NSAID alone	3	1	7	6
Prednisolone (<10mg/day)	3	2	0	0
Penicillamine (500mg/day)	1	1	0	0
Prednisolone (<7.5mg/day) + penicillamine (<500mg/day)	2	1	0	0
Prednisolone (5mg/day) + chloroquine (250mg/day)	0	0	1	0
Sodium aurothiomalate total dose 600mg	0	0	1	0

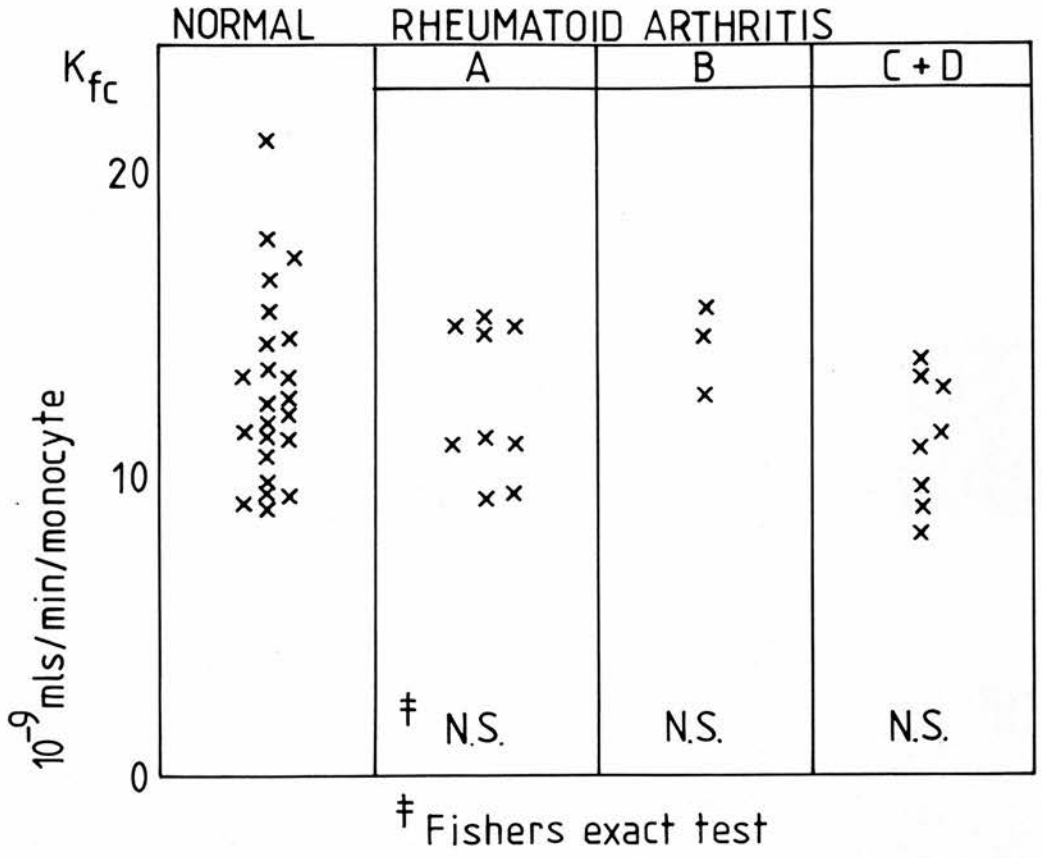
* Patient group A = active vasculitis
B = Inactive vasculitis
C = Multiple nodules
D = Miscellaneous complications

RATE CONSTANT (Kfc) FOR PHAGOCYTOSIS OF IgG COATED C. ALBICANS BY MONOCYTES FROM NORMAL CONTROLS AND RA PATIENTS.

No significant differences in the rate of Fc receptor mediated phagocytosis were found between the normal controls and any of the patients studied (Fig IV.5). Thus the functional defect of phagocytosis found when serum opsonised C. albicans was used could not be attributed to a defect of Fc receptor mediated phagocytosis.

Fig IV.5 Rate constant Kfc for phagocytosis of IgG coated C. albicans by monocytes form normal controls and RA patients.

Group A = RA patients with vasculitis;
Group B = RA patients with inactive vasculitis;
Group C & D = RA patients with multiple nodules or miscellaneous complications and diseases associated with RA.



2.2 RATE CONSTANT (Kc) FOR PHAGOCYTOSIS OF COMPLEMENT (C3b) COATED S. CEREVISIAE BY MONOCYTES FROM NORMAL CONTROLS AND RA PATIENTS.

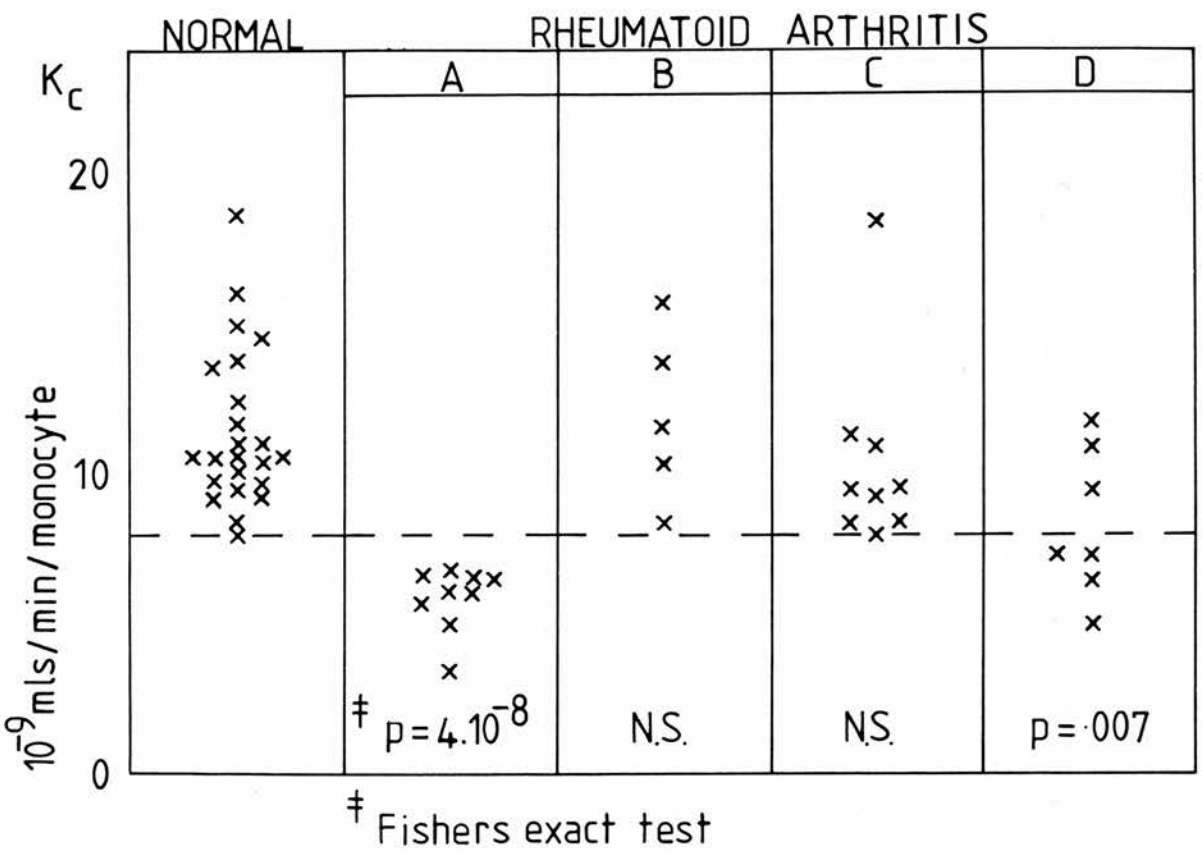
All the patients with active cutaneous vasculitis had reduced Kc compared with normal controls ($p<0.001$; Fishers one tailed exact test) while five patients with inactive vasculitis had normal Kc (Fig IV.6 - Groups A & B).

The nine patients with multiple nodules had normal Kc (Fig IV.6 - Group C), but a significant proportion (4/7) of patients with extra-articular manifestations other than vasculitis were found to have reduced Kc ($p<0.01$; Fishers one tailed exact test)(Fig IV.6 - Group D). Of these four, two had pyoderma gangrenosum, one had pericarditis, and the fourth multiple nodules and primary biliary cirrhosis. Of the three remaining patients with normal Kc, one had Feltys' syndrome and two had serious pyogenic infections.

Thus depressed rates of immune phagocytosis by monocytes appeared to be due to an abnormality of "complement" rather than Fc receptor mediated ingestion. Additionally, although the abnormality was consistently present in patients with vasculitis, it was also present in patients with other serious extra-articular manifestations of RA. To confirm the preliminary observation that the abnormality of phagocytosis was transient, serial studies were carried out on five patients.

Fig IV.6 Rate constant Kc for phagocytosis of "complement" opsonised S. cerevisiae by monocytes from normal controls and RA patients.

Group A = RA patients with active vasculitis
Group B = RA patients with inactive vasculitis
Group C = RA patients with multiple nodules
Group D = RA patients with miscellaneous complications and diseases associated with RA.

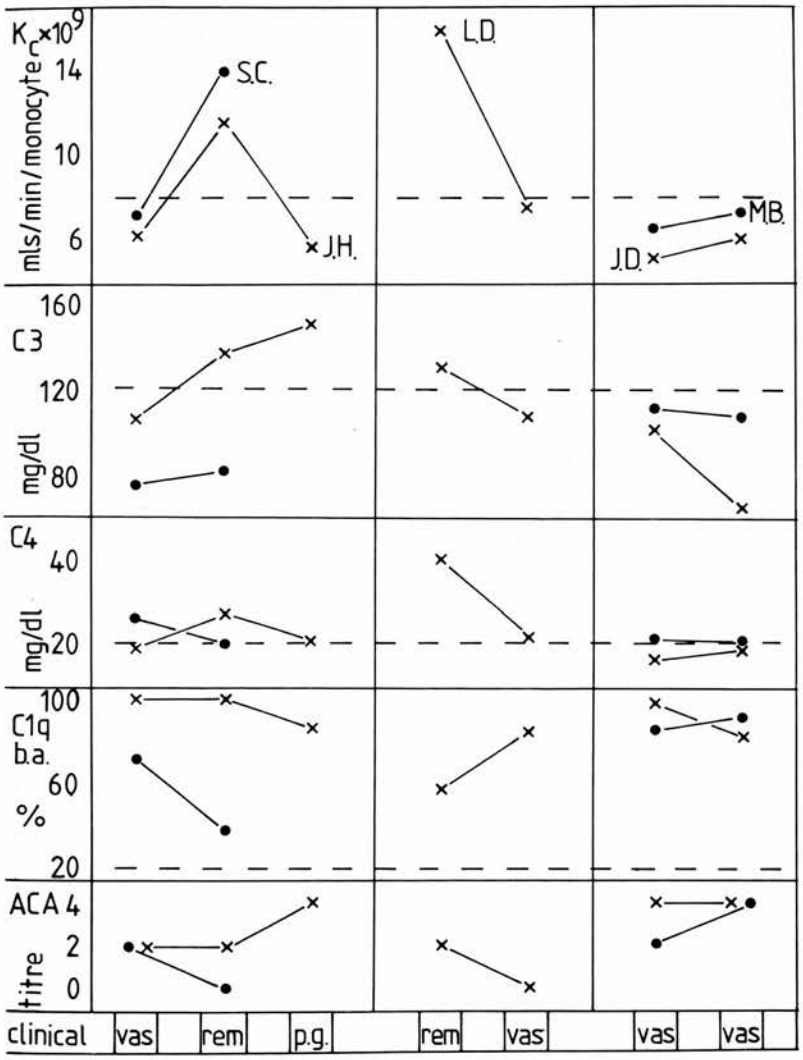


RESULT OF SERIAL STUDIES OF MONOCYTE PHAGOCYTOSIS IN PATIENTS WITH DEPRESSED RATES OF "COMPLEMENT" RECEPTOR MEDIATED PHAGOCYTOSIS

The results of serial studies are shown in Fig IV.7. Two patients (S.C. & J.H.) were studied initially when vasculitis was active and again later after cessation of vasculitis. In both of these patients Kc returned to the normal range during remission of the vasculitis. Patient J.H. later developed pyoderma gangrenosum during which Kc again fell below normal levels. A third patient (L.D.) was studied fortuitously before development of vasculitis and later during vasculitis - again depression of Kc was seen only during the episode of vasculitis. Two patients with vasculitis (J.D. & M.B.) each had further episodes of vasculitis some weeks later and were restudied. On both occasions Kc was found to be reduced. It was therefore concluded that the reduction in Kc was transient and temporally related to the inflammatory episode.

Since vasculitis may be associated with the appearance of complement activating immune complexes, the phagocytic data was examined for correlations with serum complement (C3 & C4) concentrations and levels of Clq binding activity. Also to examine the possibility that receptor blockade by immune complexes might be occurring, cytocentrifuge smears and washed cell suspensions of mononuclear cells from four patients with reduced Kc were examined by direct immunofluorescence for the presence of intracytoplasmic immune complexes and membrane associated immune complexes respectively.

Fig IV.7 Serial studies of serum complement levels (C3 & C4), Clq binding activity (Clqba), anti-complementary activity (ACA) and phagocytosis by monocytes from RA patients with depressed rates of phagocytosis of "complement" coated S. cerevisiae (Kc).



vas = vasculitis

rem = remission

p.g. = pyoderma gangrenosum

CORRELATION OF RATES OF "COMPLEMENT" RECEPTOR MEDIATED PHAGOCYTOSIS WITH SERUM COMPLEMENT (C3, C4), ANTICOMPLEMENTARY ACTIVITY & Clq BINDING ACTIVITY.

Patients with vasculitis and low Kc had significantly lower mean serum C3 levels ($p<0.02$; Students t-test) and C4 levels ($p<0.01$) and higher titres of anticomplementary activity (ACA) ($p<0.001$; Fishers one tailed exact test) than patients with normal Kc (Fig IV.8), although many of the latter also had subnormal complement levels and elevated ACA titres. It is also clear that mean C3 levels in patients with vasculitis are biased by a single low value and the numbers are small. Furthermore, serum ACA titres and mean serum C3 and C4 concentrations in the four RA patients with reduced Kc who did not have vasculitis were no different from RA patients with normal Kc (Fig IV.8). Thus, reduction of Kc was not invariably accompanied by evidence of complement activation and low serum complement levels.

Results of Clq binding activity were available from 29 studies (22 patients). Although overall, depression of Kc correlated with high levels of Clq binding activity, it is clear that some patients with gross elevation of Clq binding had normal Kc (Fig IV.9).

Values were also available for the five patients who were studied serially, and this data was also examined individually for correlations between Kc, serum complement and tests for immune complexes (Fig IV.7). In patient S.C. remission of vasculitis was accompanied by a rise in Kc and a concomitant fall in both Clq

binding activity and serum anti-complementary activity. Serum C3 and C4 did not change significantly. Patient J.H. was first studied during active vasculitis and her subsequent clinical improvement was marked by a rise in Kc, serum C3 and C4. However, Clq binding activity and anti-complementary activity remained high and 16 weeks later this patient developed pyoderma gangrenosum. The appearance of pyoderma gangrenosum was accompanied by a further fall in Kc and a rise in anti-complementary activity. In view of this the further rise in serum C3 levels was unexpected but may reflect an acute phase response of C3. In patient L.D. the appearance of vasculitis was accompanied by a marked fall in Kc, serum C3 and serum C4 and rise in Clq binding activity. However titres of anti-complementary activity surprisingly fell to zero. In patients M.B. and J.D. with recurrent vasculitis Kc and serum C3 levels remained depressed and titres of anti-complementary activity and Clq binding activity remained elevated. Serum C4 levels remained unchanged.

In general therefore, vasculitis was accompanied by evidence of increased complement consumption and increased levels of circulating immune complexes and was associated with reduced C3b receptor mediated phagocytosis.

IMMUNOFLUORESCENCE.

Direct immunofluorescence on mononuclear cells from four patients with reduced Kc failed to reveal either cytoplasmic or membrane bound complement or immunoglobulin.

Fig IV.8 Results of serum C3, C4 levels and anti complementary activity (ACA) in RA patients.

(——— indicates mean value)

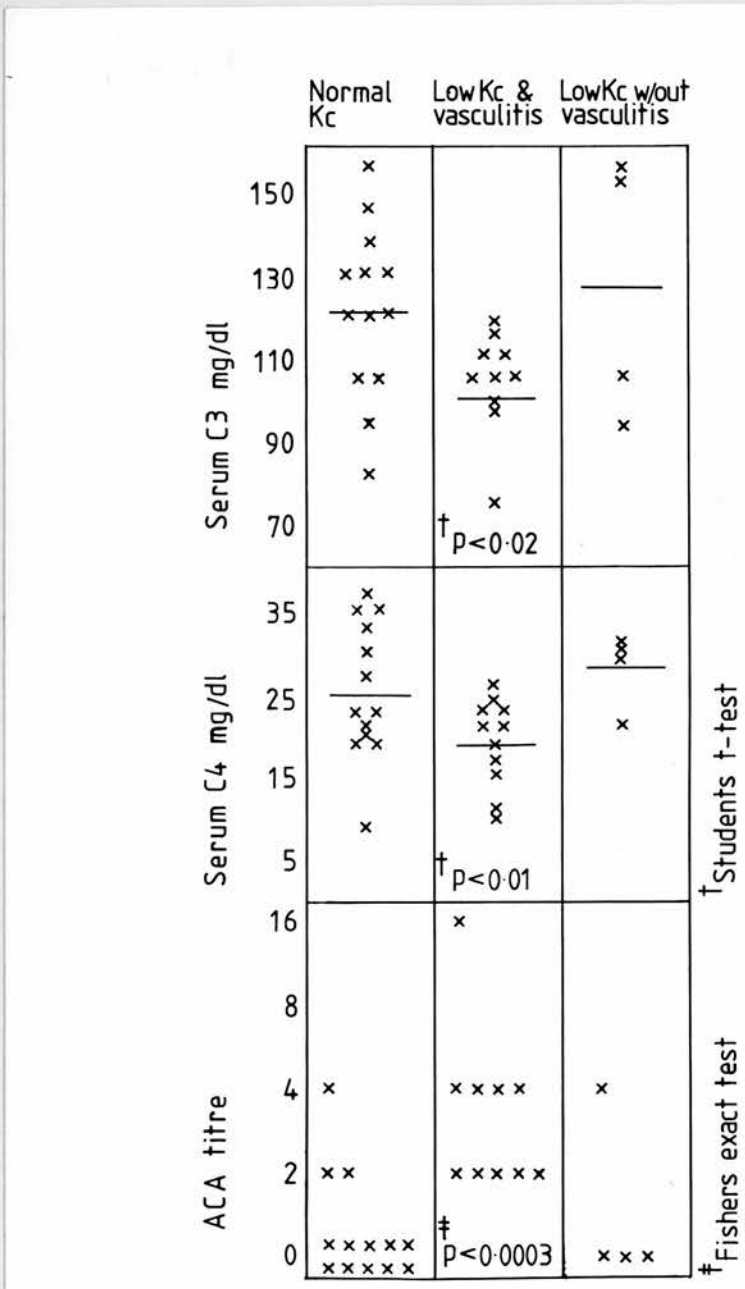
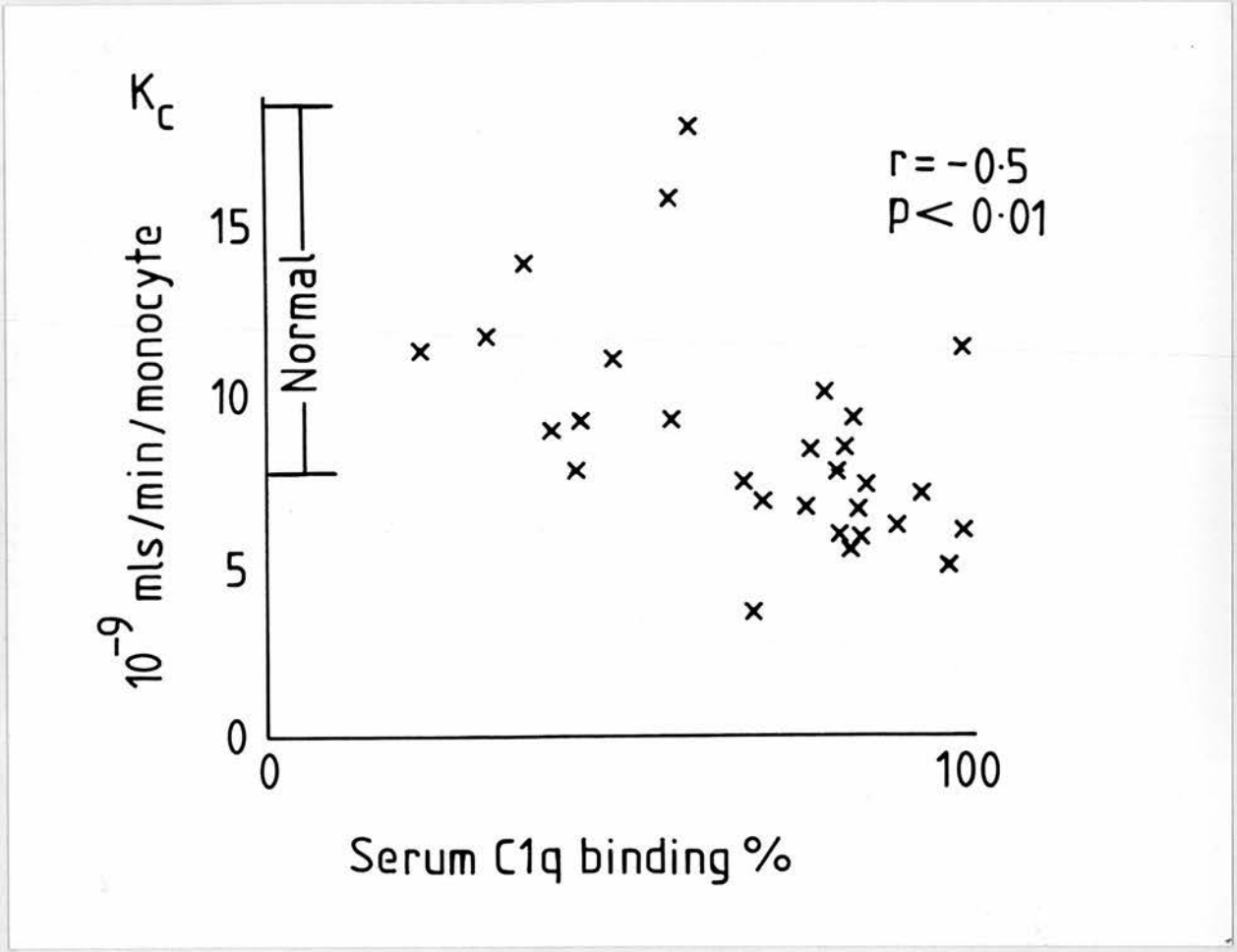


Fig IV.9 Correlation of Clq binding activity (Clqba) with rate of phagocytosis of "complement" coated S. cerevisiae (Kc).



3.0 EFFECT OF RA SERUM ON RATES OF PHAGOCYTOSIS BY NORMAL MONOCYTES.

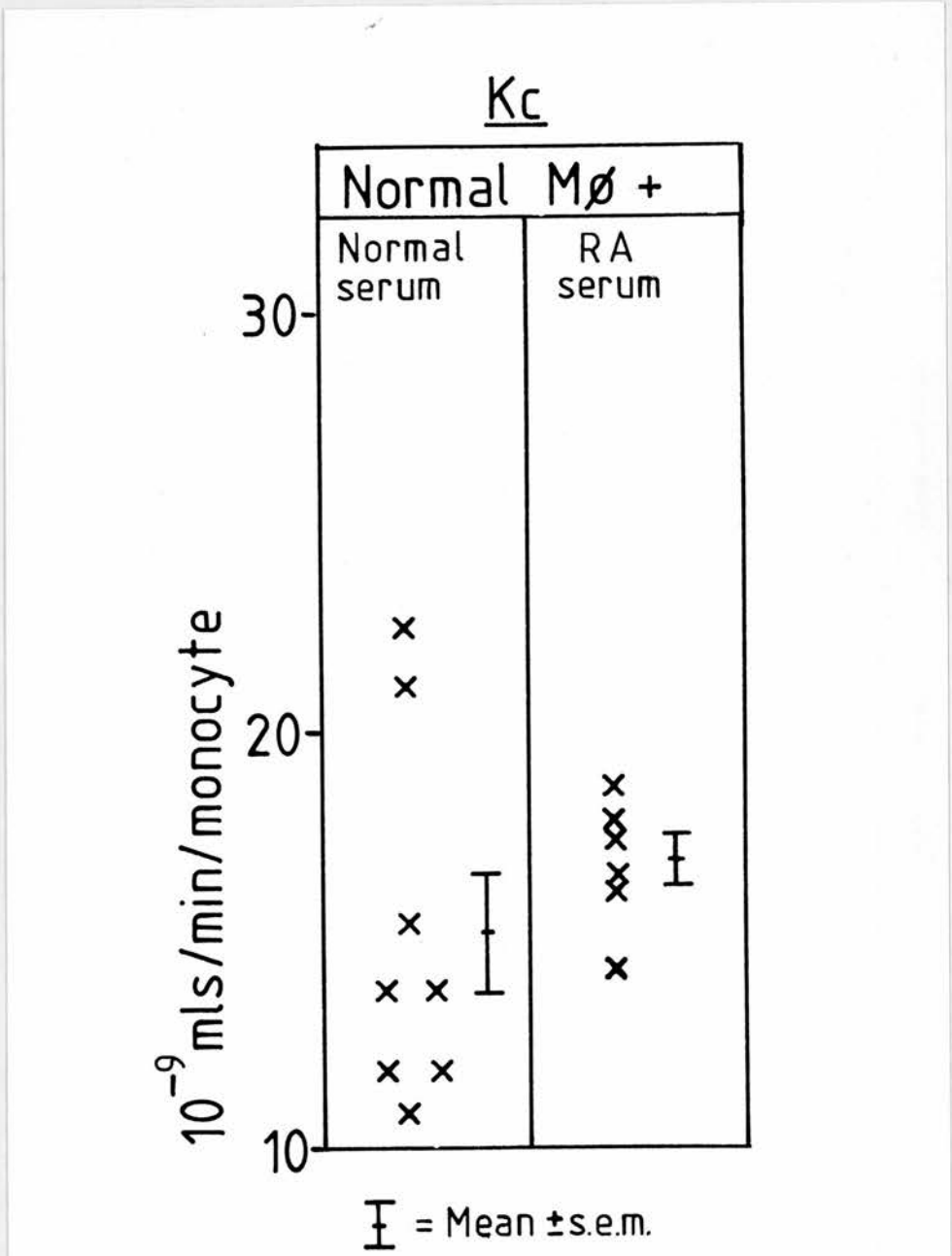
Serum drawn at the time of each phagocytic assay was stored in liquid nitrogen for studies of the effect of patients sera on normal monocytes. Normal control sera were stored similarly.

To examine the possibility that immune complexes or other serum factors were responsible for reduction of "complement" receptor mediated monocyte phagocytosis, normal monocytes (one donor) were preincubated for 60 minutes at 37°C with either 15% normal control serum (n=8) or 15% serum from RA patients (n=6) with reduced Kc. The phagocytic rate constant Kc was then determined as before.

Serum from RA patients with low Kc did not inhibit "complement" receptor mediated phagocytosis by normal monocytes (Fig IV.10).

Fig IV.10 Effect of serum from RA patients with vasculitis on "complement" receptor mediated phagocytosis by normal monocytes.

Preincubation of normal monocytes for 60 minutes with RA serum did not depress phagocytosis of "complement" opsonised S. cerevisiae:



4.0 RELATION BETWEEN RATES OF C3b RECEPTOR MEDIATED PHAGOCYTOSIS AND THE CYTOCHEMISTRY OF LARGE MONONUCLEAR CELLS.

After separation of mononuclear cells in each experiment monocytes were enumerated routinely using the Coulter counter except in 15 experiments where a differential count was also performed using NSE staining. Also, to exclude the possibility of significant contamination by neutrophil granulocytes, AS-D chloroacetate esterase staining was carried out on 8 normal control subjects and 14 RA patients.

Since no direct evidence of monocyte receptor blockade by immune complexes was found which could account for impaired "complement" receptor phagocytic function an alternative hypothesis was considered; viz. that changes in the composition of blood subpopulations of monocytes were occurring and that increased numbers of immature monocytes with poorly expressed CR1 or CR3 receptors, but normal Fc receptors, were appearing in the circulation. To examine this possibility the cytochemical characteristics of the mononuclear cells from these patients were reviewed, and the assumption that all large mononuclear cells (>250 cubic microns) were non specific esterase positive monocytes was re-examined.

AS-D chloroacetate esterase staining of mononuclear cells showed few neutrophil granulocytes (Table 6.5), and there was no significant difference between controls, RA patients with normal Kc and those with reduced Kc. Thus there was no evidence of significant contamination by neutrophil polymorphs.

Large (>250 cub microns) mononuclear cells were more frequent in RA patients than in normal controls ($p<0.001$; students t-test), but there was no significant difference between RA patients with normal Kc and those with low Kc ($p<0.5$) (Table 6.6). Thus using size as the criterion for counting monocytes there was a significant increase in the relative number of monocytes in RA patients, but no difference between those with low or normal Kc.

Although in RA patients with normal Kc the proportion of mononuclear cells staining with NSE correlated closely with the proportion of large cells (>250 cub microns) (Fig IV.3), in RA patients with low Kc this was not the case. In these patients the percentage of monocytes determined by NSE staining (19.0 +/- 5.3) was significantly less than that obtained by Coulter sizing (33.9 +/- 7.0) ($p<0.001$; students t-test). Furthermore the rate constant Kc for "complement" receptor mediated phagocytosis declined in direct proportion to the discrepancy between the Coulter and NSE differential counts (Fig IV.11) while Kfc remained unchanged.

These observations suggested that the reduction in Kc was due to the presence of increased numbers of "NSE-negative" mnocytes with reduced "complement" receptor but normal Fc receptor phagocytic function. To test this possibility the composition of the mononuclear cell population was examined prospectively in a group of normal controls, a group of RA patients and a group of RA patients with vasculitis or other extra-articular disease. In each group the %age of large (>250 cub microns) cells, %age of NSE +ve cells, %age of cells phagocytosing IgG coated yeast and %age of

cells phagocytosing complement coated yeast were estimated microscopically.

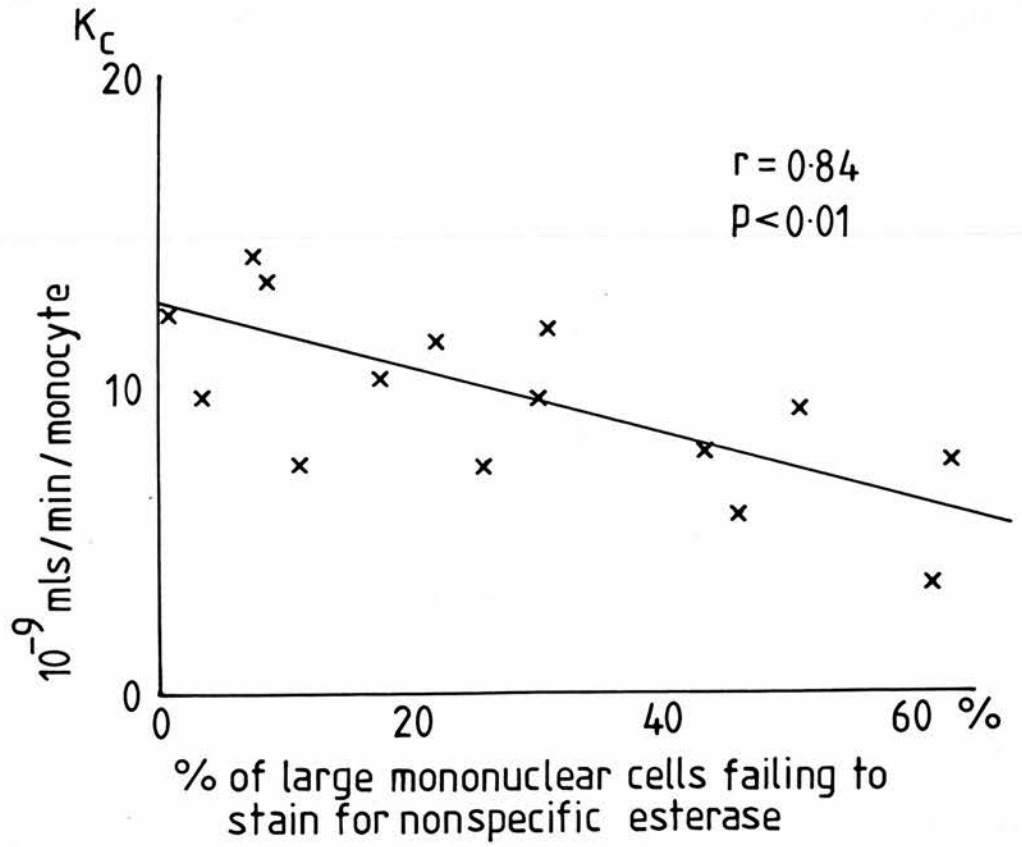
Table 6.5 Percentage of neutrophil polymorphs (PMN) contaminating mixed mononuclear cell preparations estimated by AS-D chloroacetate esterase staining.

	n	%PMN (range)
Normal subjects	8	0.5 (0 - 2.0)
RA - normal Kc	9	0.4 (0 - 4.0)
RA - reduced Kc	5	1.0 (0 - 3.0)

Table 6.6 Percentage of monocytes (mononuclear cells > 250 cubic microns) in mixed mononuclear cell preparations estimated by Coulter "sizing".

	n	%monocyte (+/- S.D.)	Range
Normal subjects	13	24.2 +/-3.9	16.7-31.7
RA - normal Kc	17	34.8 +/-8.0	27.4-59.0
RA - reduced Kc	15	33.9 +/-7.0	21.4-44.2

Fig IV.11 Correlation between reduction in Kc and percentage of large monocuclear cells (>250 cub microns) failing to stain for non-specific esterase.



5.0 COMPOSITION OF THE MONONUCLEAR CELL POPULATION IN NORMAL CONTROLS & RA PATIENTS

Controls:

Eleven healthy hospital employees (8 female, 3 male) mean age 38years (range 21-54).

RA patients:

Rheumatoid arthritis "RA" group - 12 patients with classical or definite RA (7 female, 5 male) mean age 60 (range 42-79).

Rheumatoid vasculitis "RV" group - 11 patients with classical or definite RA with extra-articular manifestations including active or recently active vasculitis (n=9); pyoderma gangrenosum (n=1); multiple nodules and primary biliary cirrhosis (n=1).

Drug therapy: Patients drug therapy is shown in Table 6.7.

Table 6.7 Drug therapy of "RA" and "RV" patients.

	Patient group	
	"RA"	"RV"
NSAID alone	9	3
Prednisolone (+/- other drugs)	2	4
Penicillamine (+/- other drugs)	1	4
Sodium aurothiomalate (+/- other drugs)	1	0

INVESTIGATION OF THE COMPOSITION OF THE MONONUCLEAR CELL POPULATION IN CONTROLS AND RA PATIENTS

Mononuclear cells were separated from venous blood obtained from the above subjects and the concentration of monocytes determined using both NSE staining and Coulter sizing.

The %age of monocytes capable of phagocytosing either IgG or "complement" coated yeast were estimated by incubating the mononuclear cells with yeast in suspension for 40 minutes, after which cytocentrifuge slides were prepared and stained with NSE. The number of phagocytic cells was then estimated microscopically from a count of at least 200 cells as described above (p200). The typical microscopic appearance of phagocytic cells is shown in Fig IV.12.

There was no significant difference (Students t-test) between the three groups in the percentage of monocytes in the mononuclear cell population as estimated by NSE staining or Coulter sizing, although the latter consistently but systematically overestimated the percentage of monocytes by 3-4% (Table.6.8). Thus there was no evidence in this study of a greater proportion of large NSE negative cells in the "extra-articular manifestations" (RV) group than in the uncomplicated RA patients or controls. However, in the RV group there was a significant reduction in the number of monocytes phagocytosing "complement" coated yeast compared to the %age phagocytosing IgG coated yeast, while in the controls and uncomplicated RA patients there was no difference (Table 6.9).

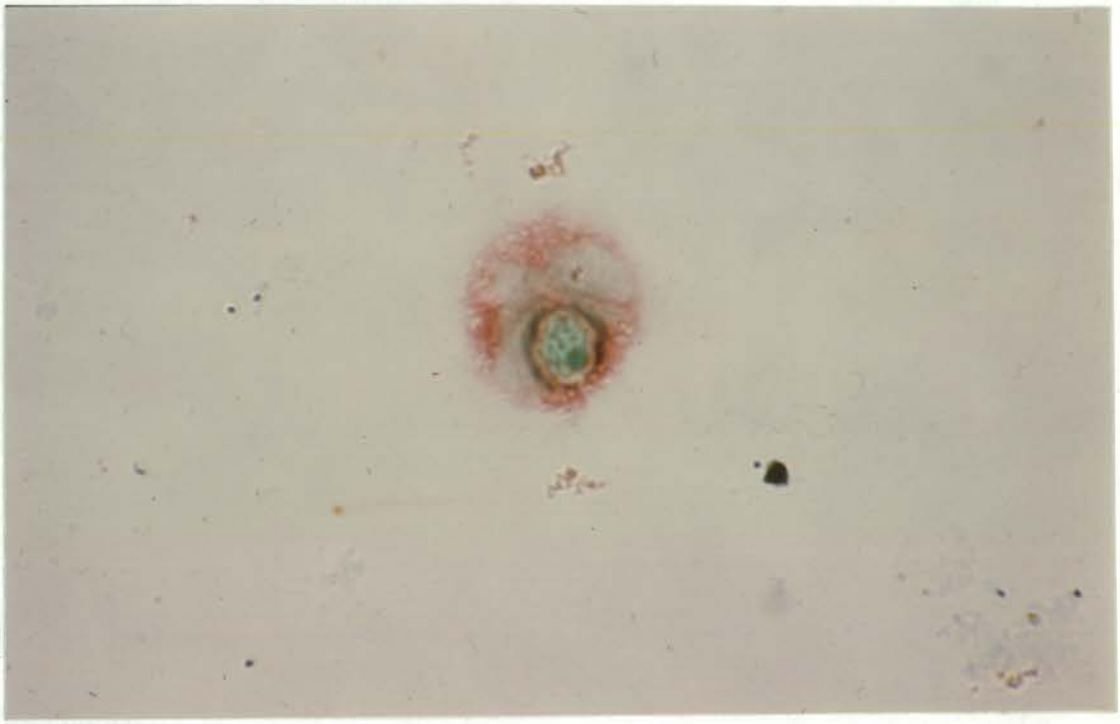
These results demonstrate that the reduction in Kc seen in

patients with rheumatoid vasculitis is due to the presence of NSE positive monocytes with phagocytic Fc receptors but functionally inactive or absent "complement" receptors. Thus although the results suggest a change in the composition of the peripheral mononuclear cell population in vasculitis they do not support the hypothesis that there are NSE-negative, Fc-bearing, mononuclear phagocytes with functionally inactive complement receptors.

Fig IV.12 Microscopic appearance of monocyte containing phagocytosed yeast. Cells are stained with NSE and counterstained with methyl green.

Top - Strongly staining NSE-positive monocyte containing a single yeast.

Bottom - Weakly staining NSE-positive monocyte containing two ingested yeast, with three adherent yeasts on its surface. Two yeast are lying free at bottom.



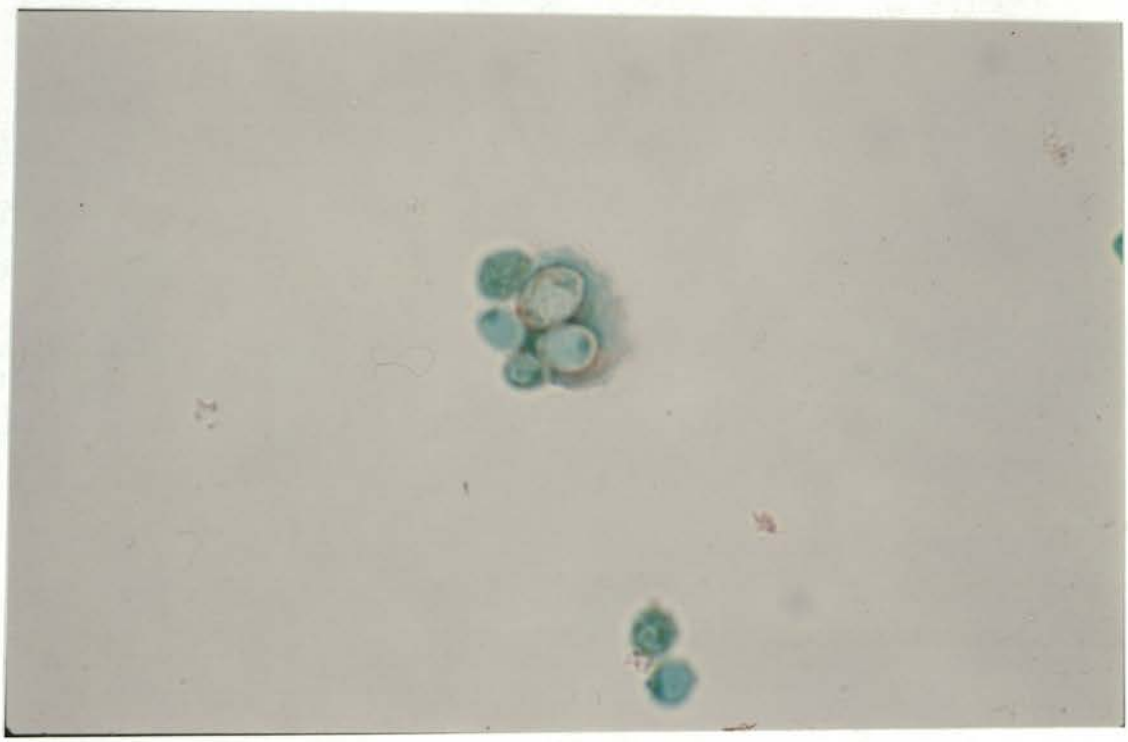


Table 6.8 Percentage (+/- S.D.) of monocytes in mixed mononuclear cell populations estimated by NSE staining or Coulter "sizing".

		Mononuclear cells	
	n	% NSE +ve	% >250 cub microns
Normal controls	11	27.0 +/-6.2	32.2 +/-7.8
"RA" patients	12	31.3 +/-9.1	35.1 +/-9.3
"Rv" patients	11	27.8 +/-7.9	32.6 +/-8.6

Table 6.9 Percentage (+/- S.D.) of cells in mixed mononuclear cell populations phagocytosing IgG coated yeast (%Fc positive) or "complement" coated yeast (%CR positive).

A significant reduction in %age of mononuclear cells phagocytosing "complement" coated yeast is seen in the "RV" group of patients:

		Mononuclear cells		
	n	% FcR +ve	% CR +ve	FcR vs CR*
Normal controls	11	21.1 +/-3.7	21.1 +/-4.0	N.S.
"RA" patients	12	22.2 +/-5.0	22.0 +/-6.3	N.S.
"RV" patients	11	20.9 +/-5.6	13.9 +/-4.0	$p<0.001$

*Students t-test.

INVESTIGATION OF THE COMPOSITION OF THE ADHERENT CELL POPULATION IN CONTROLS AND RA PATIENTS

Having established that there is a reduction in numbers of monocytes capable of phagocytosing "complement" coated yeast in the mixed mononuclear cell population obtained from patients with extra-articular manifestations ("RV" patients), limited preliminary studies have been performed in four "RV" subjects and five of the above "RA" patients to determine whether these cells can be isolated in the adherent cell population.

Mononuclear cells were plated onto glass coverslips and cultured as described above (p201). After removal of nonadherent cells, the adherent population was incubated with either IgG or complement coated yeast with rocking for 40 minutes and the %age of phagocytic cells estimated after staining with NSE. Some coverslips were not incubated with yeast but stained with NSE to determine the %age of NSE positive cells.

The percentage of NSE positive mononuclear cells and the percentage of cells phagocytosing either IgG coated (FcR +ve) or "complement" coated yeast (CR +ve) in both the mixed mononuclear cell population and the adherent cell population are shown in Table 6.10. Although there is a marked reduction in the percentage of CR-positive cells compared to the number of FcR-positive cells in the mixed mononuclear cell population from four "RV" patients, there is no such difference in the adherent cell population. Mononuclear cells from 5 control "RA" patients showed no reduction in numbers of CR-positive cells in either the mixed mononuclear

cells in suspension or in the adherent cell population. Thus it appears from these preliminary results that the FcR-positive, CR-negative cells are not glass adherent and are therefore lost during the adherence procedure. Studies of cells from further patients will be required to establish this point.

Table 6.10 Comparison of mean (+/- S.D.) percentages of NSE positive, FcR positive and CR positive cells in the mixed mononuclear cell population and the adherent cell population.

	Mixed mononuclear cells in suspension			
	n	%NSE +ve	%FcR +ve	%CR +ve
"RA" patients	5	36.7 +/-10	27.2 +/-3	27.1 +/-6
"RV" patients	4	26.5 +/-5	24.3 +/-8	15.5 +/-3

	Adherent mononuclear cell population			
	n	%NSE +ve	%FcR +ve	%CR +ve
"RA" patients	5	83.8 +/-5	77.8 +/-10	84.0 +/-7
"RV" patients	4	73.3 +/-17	75.9 +/-11	85.1 +/-9

SECTION V: RESULTS OF STUDIES ON PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (S.L.E.).

1.0 RATES OF PHAGOCYTOSIS OF YEAST PREOPSONISED WITH EITHER "COMPLEMENT" OR IMMUNOGLOBULIN (IgG) BY MONOCYTES FROM NORMAL SUBJECTS AND PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS.

In the group of SLE patients studied, the rate constants for "complement" and Fc receptor mediated phagocytosis, Kc and Kfc respectively, were measured separately.

SUBJECTS.

Controls: Twenty three healthy hospital employees (13 female, 10 male), mean age 32.2 years (range 21-64).

Patients: Eighteen patients with SLE (17 female, 1 male), mean age 34.8 years (range 23-65). Seventeen patients fulfilled the ARA criteria (Cohen & Canoso 1972) for definite SLE. Details of clinical features are shown in Table 6.11. Disease activity is indicated by a "clinical disease activity score" in which each active disease manifestation is given a score of +1 (Cairns et al 1980).

SEROLOGY

Serum stored at 4°C was used to measure ANF titres and to detect antibodies to soluble cellular antigens as described above (p202-212). Results are shown in Table 6.11.

DRUG THERAPY

Drug therapy is shown in Table 6.12.

Table 6.11 Clinical and serological features of SLE patients.

TABLE 1 CLINICAL AND SEROLOGICAL FEATURES OF SLE PATIENTS

PATIENT	AGE	SEX	C1q BINDING ACTIVITY % (a)	ESR mm in 1st hour	ANF TITRE	DNA BINDING units/ml (b)	ANTIBODIES TO SOLUBLE CELLULAR ANTIGENS	ACTIVE CLINICAL FEATURES	PREVIOUS CLINICAL FEATURES	CLINICAL DISEASE ACTIVITY SCORE	PHAGOCYTIC RATE (c) CONSTANT Kc	10^{-9} ml/min/monocyte
											Kfc	Kc
1	26	F	17	6	160	16	Sm/Ro	Raynauds, malar rash, arthritis	Leucopenia, nephritis, digital pulp atrophy	3	2.2	5.7
2	56	F	94	61	320	34	Ro/La	Raynauds, transverse myelitis	Photosensitivity, arthralgia	2	13.0	7.8
3	60	F	100	136	640	34	---	Arthritis, thrombocytopenia pleurisy	Leucopenia, proteinuria	3	14.8	15.0
4	33	F	33	62	320	11	---	Raynauds, neuropsychiatric syndrome, thrombocytopenia, proteinuria	Arthritis, vasculitis, myocarditis pleurisy, leucopenia, circulating anticoagulant	4	4.8	4.0
5	50	F	83	16	80	28	Ro	Arthritis, skin rash, myalgia	Glomerulonephritis, buccal ulceration	3	11.0	6.5
6	54	F	52	107	2560	19	Ro/La	Raynauds, leucopenia, arthritis.	Epilepsy, vasculitis, autoimmune haemolytic anaemia, alopecia,myopathy	3	7.3	7.6
7	35	F	97	136	5120	85	Ro/La	Raynauds	Arthritis, skin rash, vasculitis, pulmonary fibrosis	1	9.8	9.2
8	46	M	46	52	640	24	---	Arthritis, pulmonary fibrosis	Raynauds, photosensitivity	2	13.9	5.1
9	25	F	--	2	320	1	---	Remission	Arthritis, photosensitivity, transient monoparesis	0	9.2	10.6
10	65	F	7	4	2560	24	Ro	Arthritis, confusional state	Vasculitis, pleurisy	2	10.2	7.4
11	57	F	17	33	160	16	Ro/La	Remission	Arthritis, glomerulonephritis	0	12.7	8.2
12	26	F	23	30	320	31	---	Raynauds, arthritis	Skin rash	2	12.8	8.2
13	53	F	3	14	160	11	Sm/RNP Ro/La	Remission	Raynauds, arthritis, pneumonitis pericarditis, pleurisy,	0	9.9	8.4
14	48	F	0	37	320	24	Not done	Remission	Arthritis, skin rash, vasculitis, glomerulonephritis/myelitic syndrome	0	13.0	12.9
15	48	F	--	23	160	15	RNP	Remission	Raynauds, arthritis, glomerulo-nephritis, pulmonary fibrosis, myopathy, leucopenia	0	10.4	8.6
16	25	F	--	10	6144	0	Sm	Arthritis, Raynauds		2	9.2	9.7
17	34	F	0	16	640	0	Sm, Ro/La	Epilepsy, arthritis, nasal ulceration	Pyrexia, photosensitivity, Raynauds, vasculitis.	3	14.2	4.6
18	23	F	39.2	139	640	0	Sm/RNP	Pyrexia, pleurisy, myalgia, Raynauds, arthritis	Photosensitivity, depression, auto-immune haemolytic anaemia,leucopenia	5	9.3	0.9

(Normal values: (a) < 20% (b) < 25 units/ml (c) Kc > 7.9 Kfc> 0.9)

Table 6.12 Drug therapy of SLE patients.

Patient number	Therapy A mg/day	B mg/day	C mg/day	D
1	10	400	-	-
2	10	-	-	+
3	-	-	-	+
4	15	-	-	-
5	40	-	-	-
6	-	400	-	+
7	-	400	-	-
8	7.5	400	-	+
9	-	-	-	+
10	-	-	-	+
11	7.5	-	150	-
12	-	-	-	-
13	12.5	-	-	-
14	20	-	50	-
15	10	-	-	-
16	-	-	-	-
17	40	-	-	-
18	40	-	-	+

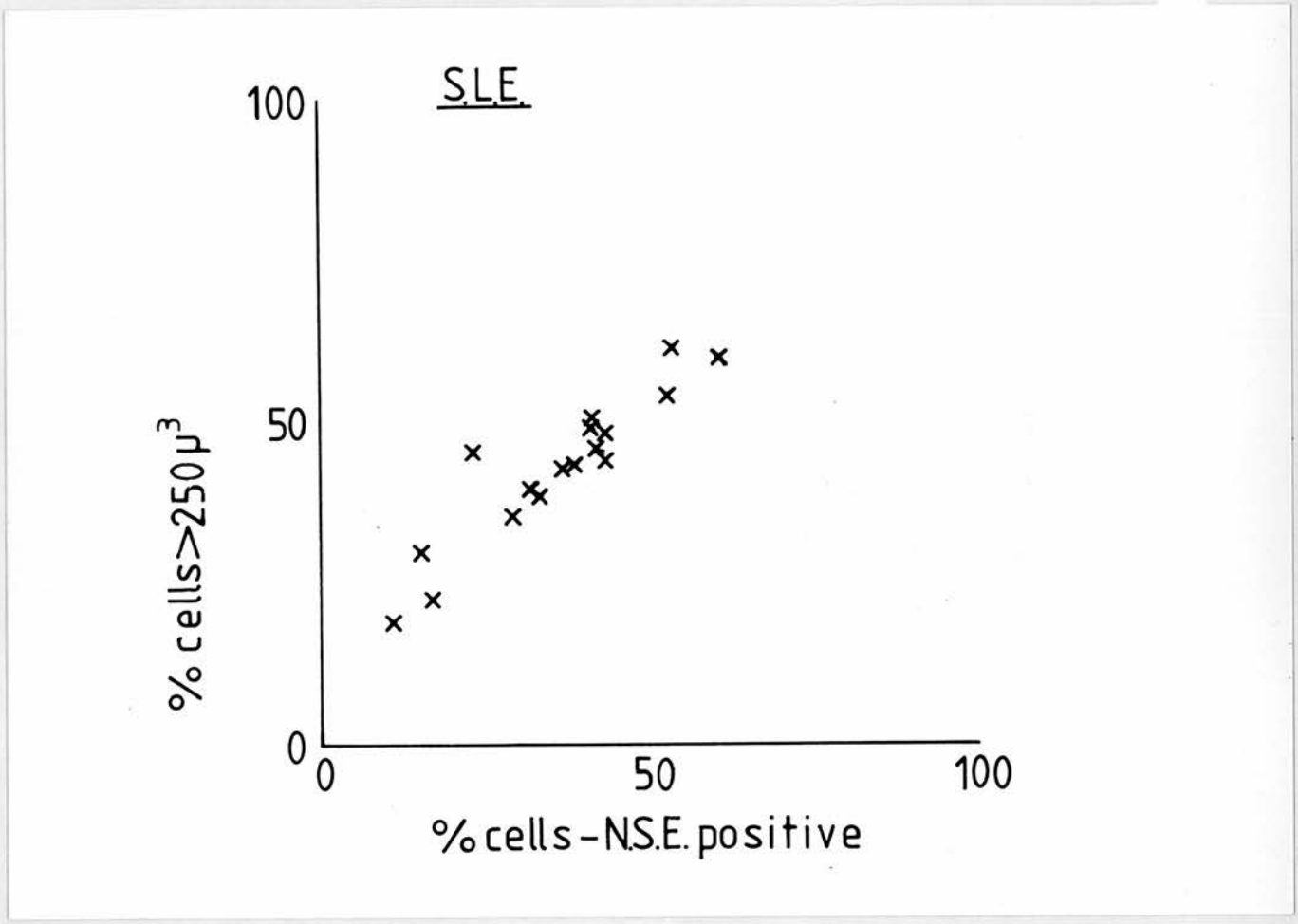
A = Prednisolone
B = hydroxychloroquine
C = azathioprine
D = NSAID

VALIDATION OF COULTER SIZING METHOD FOR PERFORMING MONOCYTE DIFFERENTIAL COUNTS ON CELLS FROM SLE PATIENTS.

To confirm that the Coulter sizing method for performing monocyte differential counts was valid for mononuclear cells from SLE patients the method was compared with NSE staining on mononuclear cells from 17 of the 18 patients studied.

A good correlation was found between the percentage of monocytes in mixed mononuclear cell populations estimated by NSE staining and the percentage estimated by Coulter sizing ($n=19$, $r=0.94$, $p<0.001$) (Fig V.1).

Fig V.1 %ages of monocytes in mixed mononuclear cell populatins from SLE patients.
Differential counts obtained by NSE staining correlate well wth the Coulter "sizing" method in which monocytes are defined as cells of volume greater than 250 cub microns (n=19;r=0.94;p<0.001).

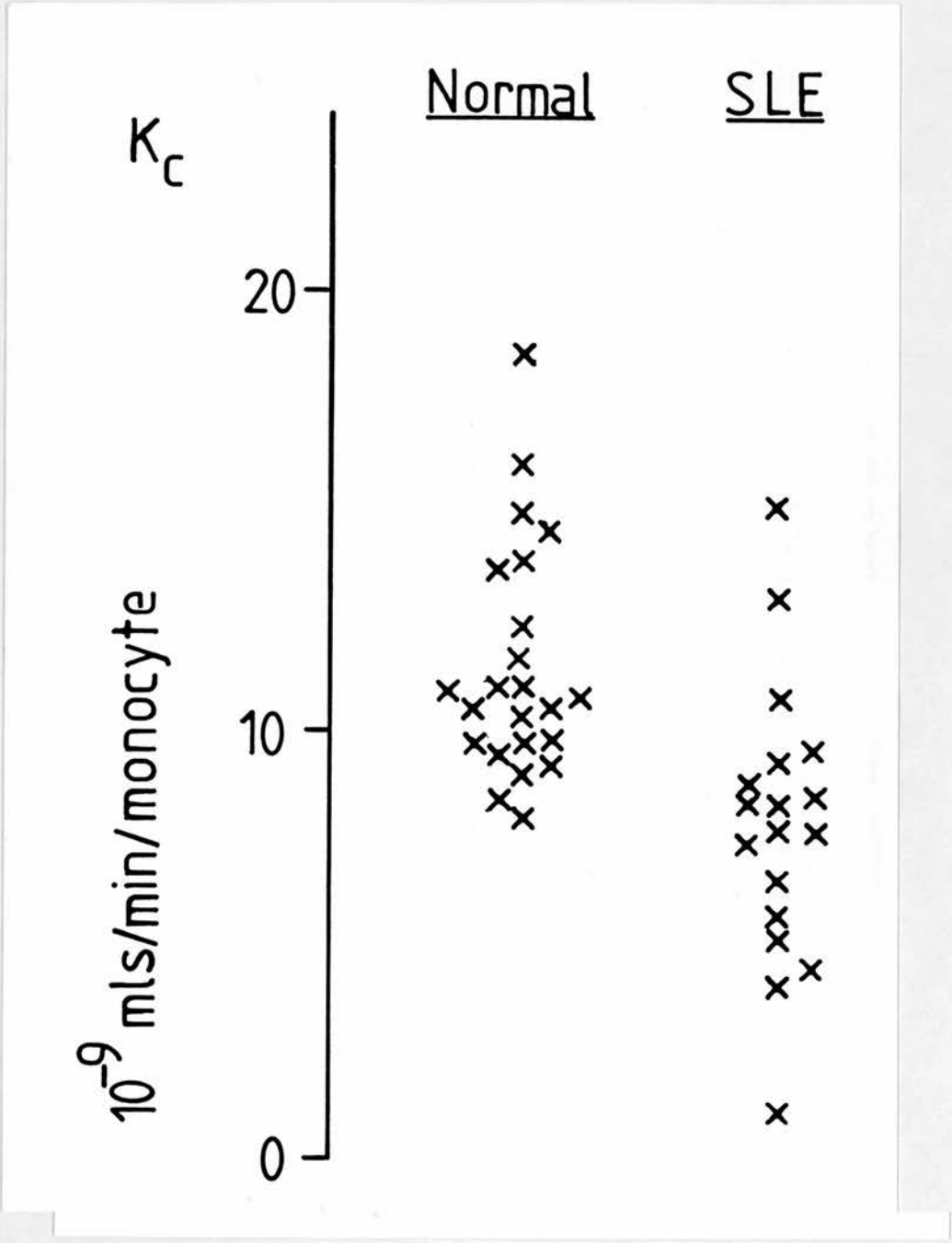


RATE CONSTANT (Kc) FOR MONOCYTE PHAGOCYTOSIS OF COMPLEMENT COATED S. CEREVISIAE BY MONOCYTES FROM PATIENTS WITH SLE.

Using complement coated S. cerevisiae rate constants for "complement" receptor mediated monocyte phagocytosis were measured as described above (p196).

Rate constants for "complement" receptor mediated phagocytosis (Kc) were reduced in 9/18 SLE patients compared to the normal controls ($p=0.0001$; Fishers one tailed exact test) (Fig V.2).

Fig V.2 Rate constant Kc for phagocytosis of "complement" coated S. cerevisiae by monocytes from normal controls and SLE patients.

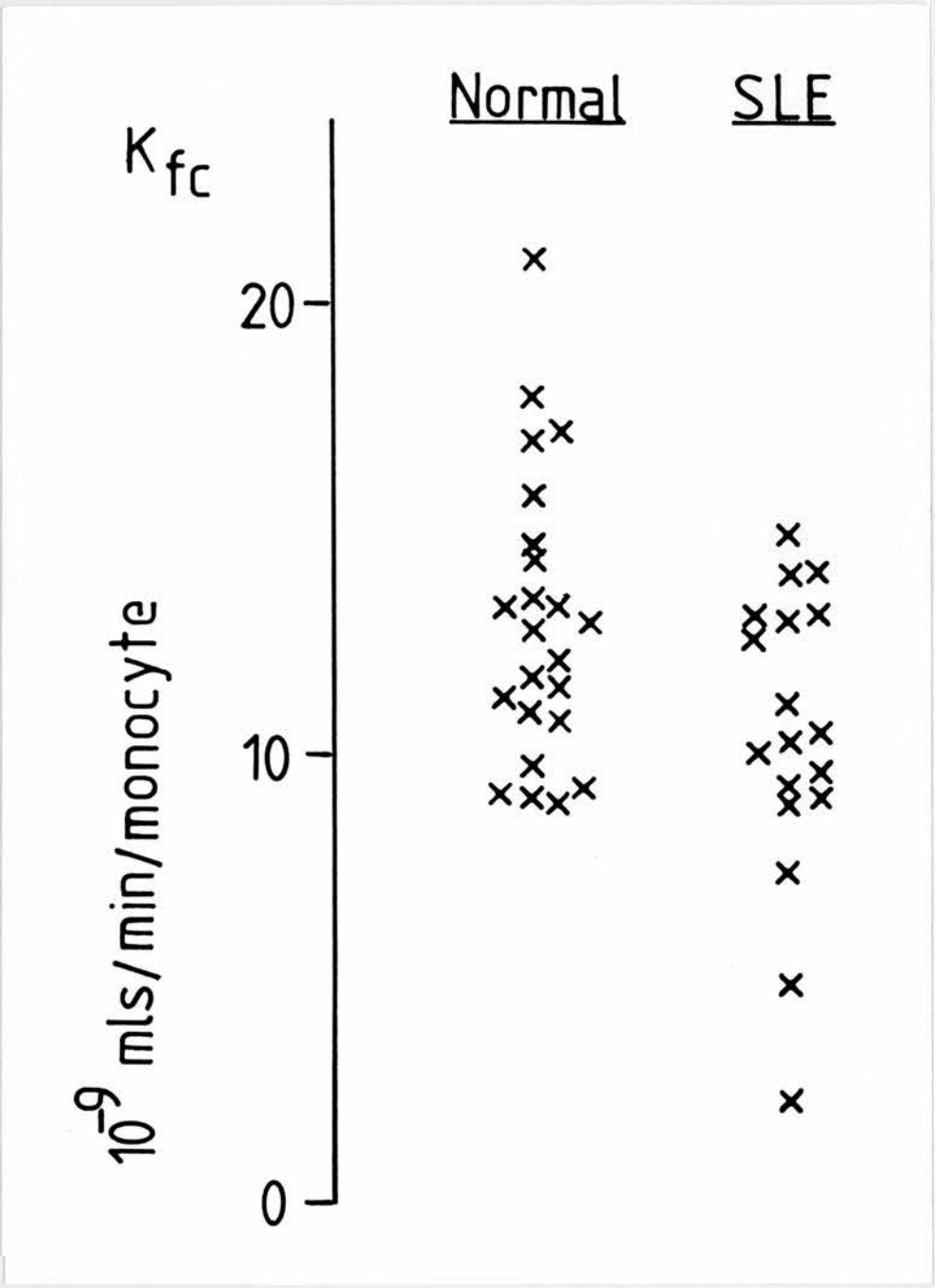


RATE CONSTANT (Kfc) FOR MONOCYTE PHAGOCYTOSIS OF IgG COATED C. ALBICANS BY MONOCYTES FROM PATIENTS WITH SLE.

Using IgG coated C. albicans the rate constants for Fc receptor mediated monocyte phagocytosis were measured as described above (p196).

Rate constants for Fc receptor mediated phagocytosis were reduced in only 3/18 SLE patients compared to the normal controls, a proportion which is not statistically significant (Fig V.3). However the magnitude of the reduction suggested that this might reflect a biological abnormality, so to determine whether it was reproducible and correlated with serological and clinical parameters, two of these three subjects were studied at intervals over an eleven month period.

Fig V.3 Rate constant Kfc for phagocytosis of IgG coated C. albicans by monocytes from normal controls and SLE patients.



RESULT OF SERIAL STUDIES OF PHAGOCYTOSIS BY MONOCYTES FROM PATIENTS WITH DEFECTS OF Fc OR "COMPLEMENT" RECEPTOR MEDIATED PHAGOCYTOSIS.

To further examine the relationship between abnormalities of monocyte phagocytosis and clinical and serological parameters, serial studies of Fc and "complement" receptor mediated monocyte phagocytosis were undertaken in two subjects A and B over a twelve month period. The results of these studies are shown in Fig V.4.

PATIENT A:

Kfc and Kc were reduced on 2 out of 5 occasions. Reduction in rates of phagocytosis correlated with disease activity but not with serological parameters. During an episode of neurological involvement, thrombocytopenia and leucopenia, both Kfc and Kc were reduced. Clinical remission and improvement in phagocytic function occurred following an increase in dosage of prednisolone to 20mg daily. Serum complement levels were persistently low, but DNA binding was normal and Clq binding activity was only slightly elevated (30%) throughout the period of study.

PATIENT B:

Kfc was reduced on 4 out of 5 occasions and showed a close correlation with serum complement levels and disease activity. On the one occasion Kfc fell within the normal range, there were no active clinical manifestations. On all other occasions, reduction of Kfc was associated with either active cutaneous rash, arthritis or leucopenia. Kc was also reduced on 4 out of 5 occasions but showed no correlation with either serological parameters or disease

activity. Clq binding activity and DNA antibody levels were normal throughout the period of study.

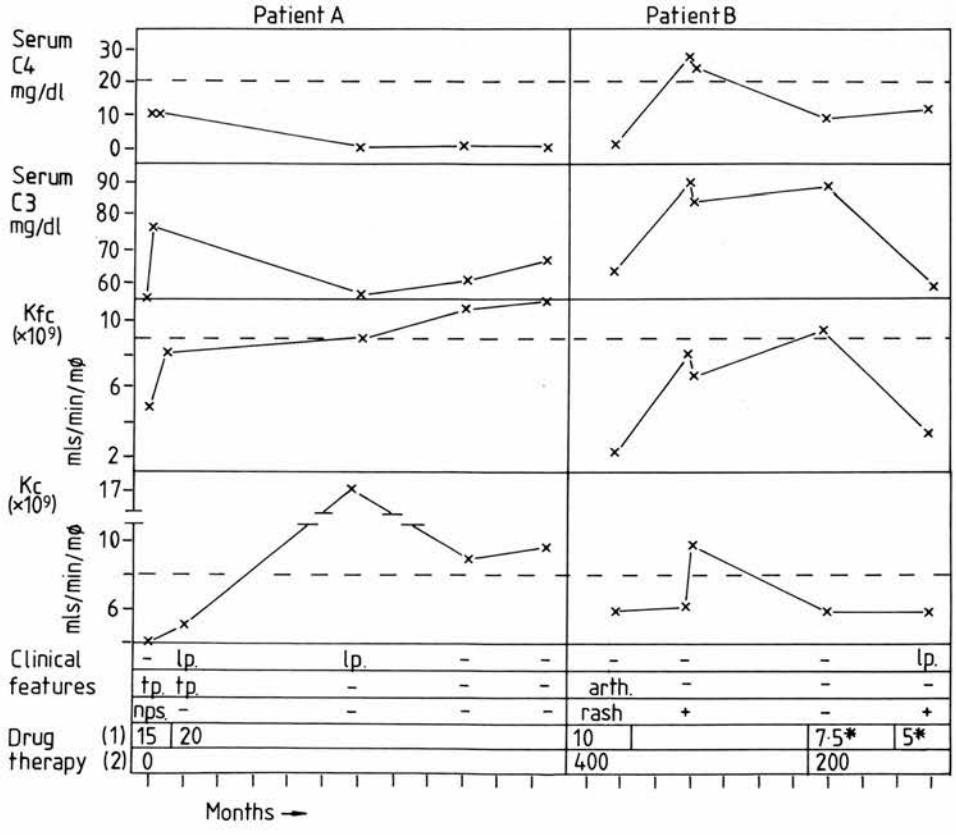
There was no relationship between phagocytic function and drug therapy and changes in the former always reflected alterations in clinical status before changes in drug therapy were instituted.

Fig V.4 Serial study of Kfc, Kc, serological and clinical parameters in patients A & B.

Key: Clinical features; lp = leucopenia < $4X10^9$ wbc/l.
tp = platelets < $100X10^9$ platelet/l.
nps = neuropsychiatric syndrome
arth = arthritis

Normal values; serum C4 > 20mg/dl
serum C3 > 120mg/dl
Kfc > $8.9X10^{-1}$ ml/min/monocyte
Kc > $7.9X10^{-1}$ ml/min/monocyte

Drug therapy; (1) = prednisolone mg/day (* alt. day)
(2) = hydroxychloroquine mg/day



CORRELATION OF RATES OF PHAGOCYTOSIS WITH SEROLOGICAL PARAMETERS

Serum drawn at the time of the phagocytic assay was stored in liquid nitrogen for subsequent measurement of serum C3 and C4 concentrations, Clq binding activity (Clqba), and DNA binding activity as described above (p202-212).

Kfc correlated with serum C3 (Fig V.5) (r_s=0.57; $p<0.01$; rank correlation) but not with serum C4 (Fig V.6) (r_s=0.33; $p>0.05$; rank correlation). Kc did not correlate with either serum C3 or C4 (Fig V.7 and 8) (rank correlation). Similarly no significant correlation was found between DNA or Clq binding activity and either Kfc or Kc (Table 6.11), and there was no significant correlation between Kfc and Kc (rank correlation).

Fig V.5 Correlation of Kfc with serum C3.

Reduction in Kfc correlates with reduced serum C3 levels (τ=0.57;p<0.01;rank correlation):

(Units of Kfc are 10^{-9} mls/min/monocyte)

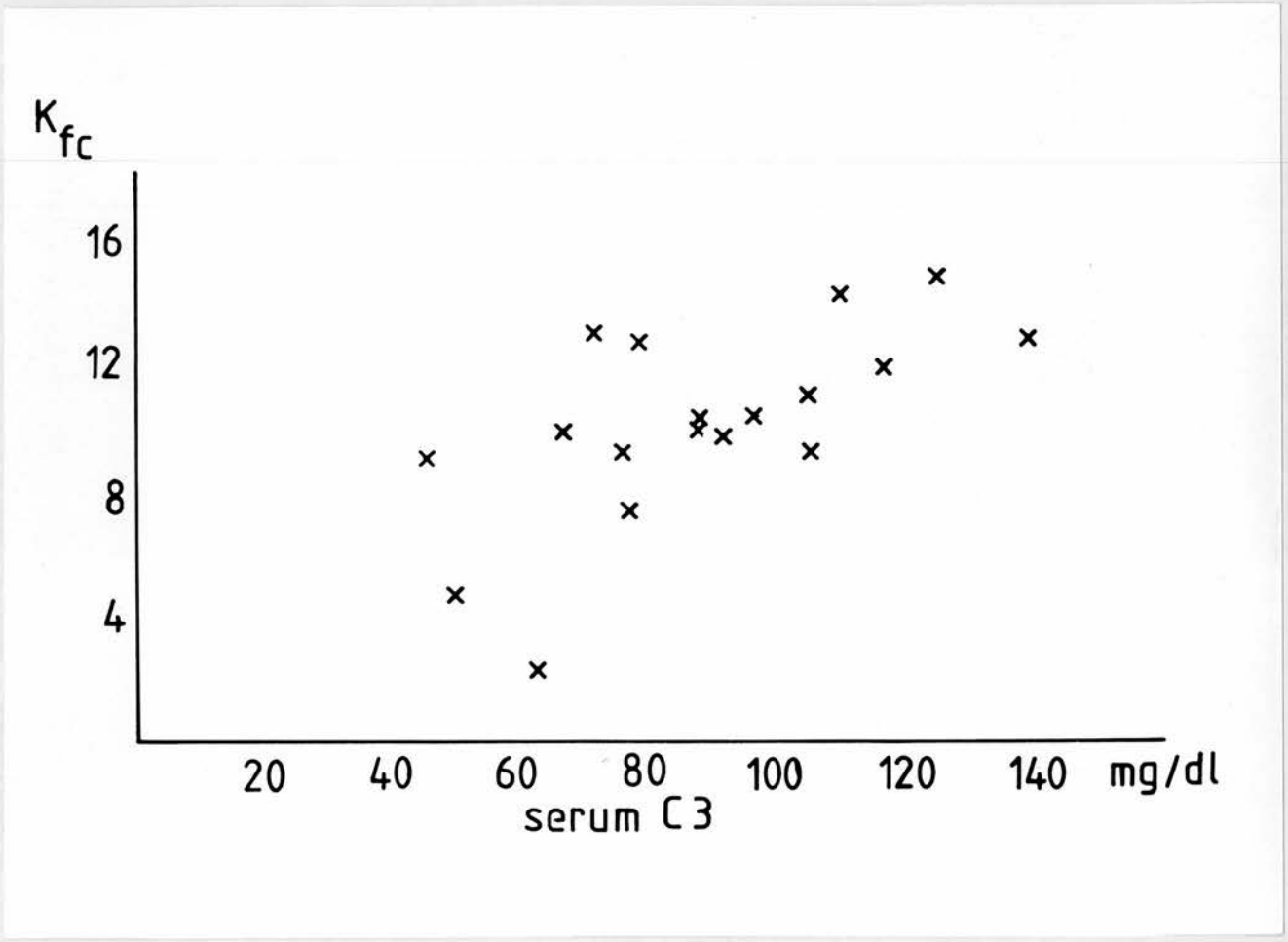


Fig V.6 Correlation of Kfc with serum C4.

Reduction in Kfc does not correlate significantly with serum C4 (rank correlation).

(Units of Kfc are 10^{-9} mls/min/monocyte)

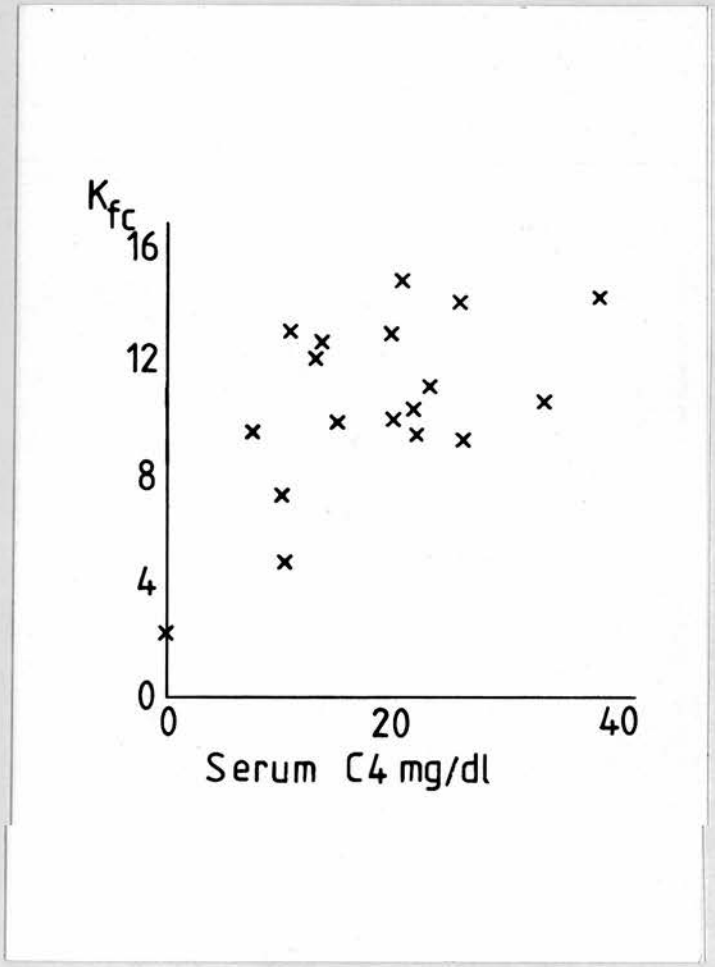


Fig V.7 Correlation of Kc with serum C3.

Reduction in Kc does not correlate significantly with serum C3 (rank correlation).

(Units of Kc are 10^{-9} mls/min/monocyte)

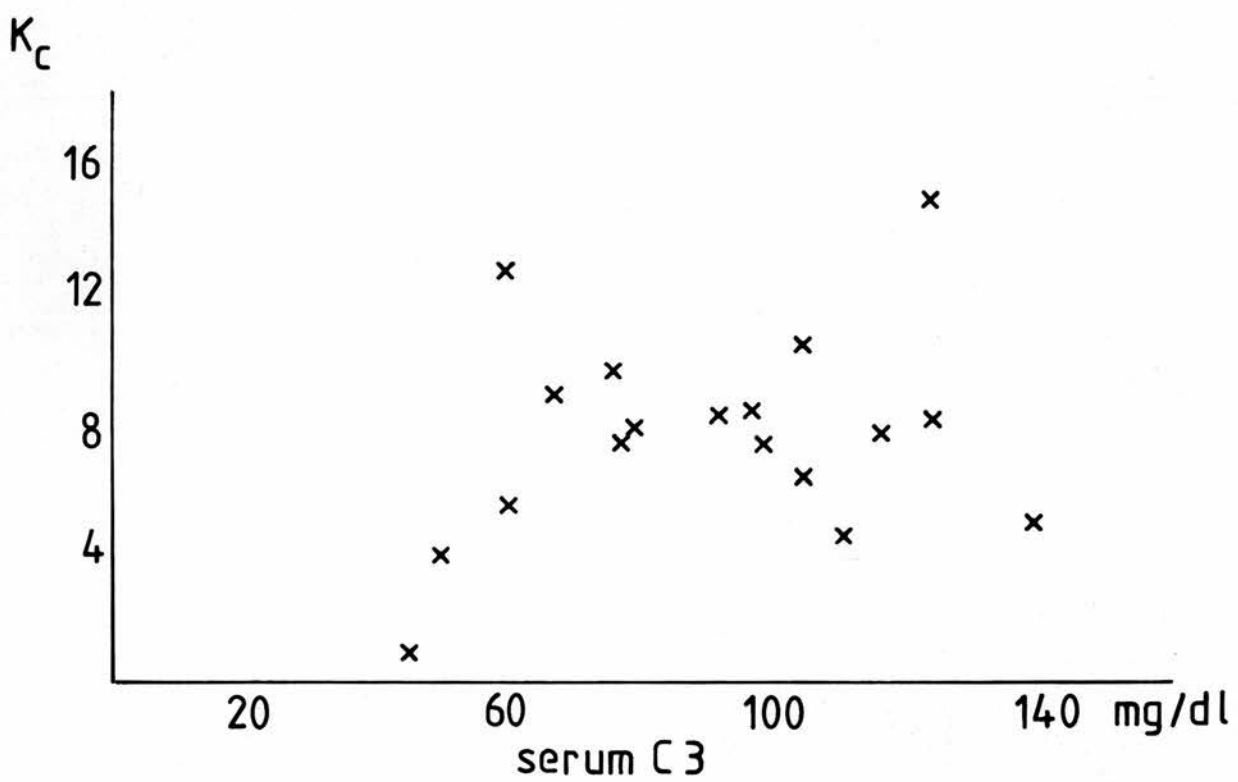
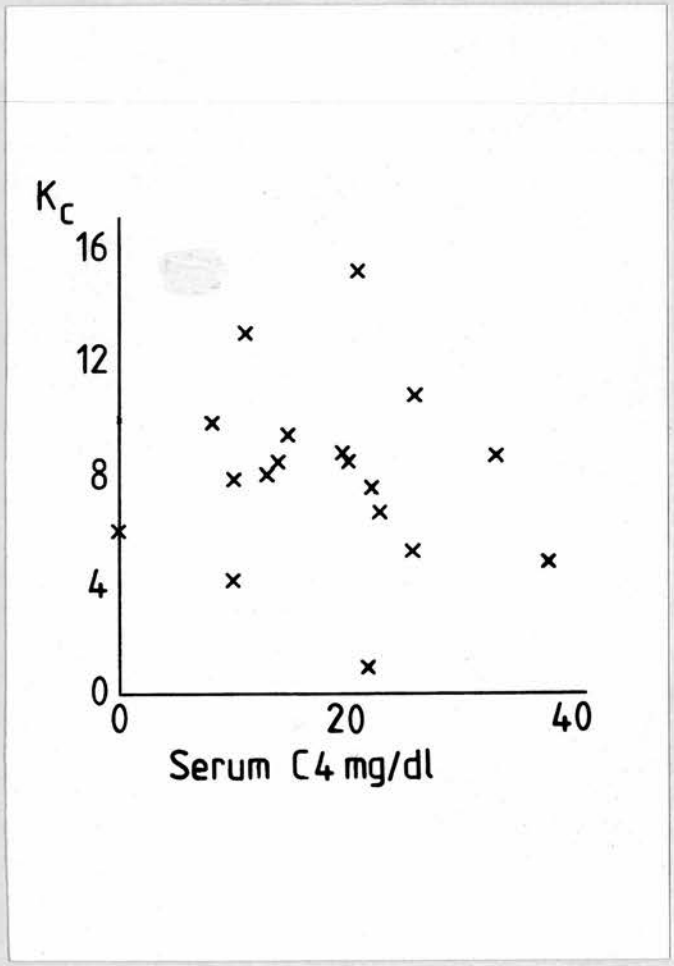


Fig V.8 Correlation of Kc with serum C4.

Reduction in Kc does not correlate significantly with serum C4 (rank correlation).

(Units of Kc are 10^{-9} mls/min/monocyte)



CORRELATION OF RATES OF PHAGOCYTOSIS WITH CLINICAL MANIFESTATIONS.

To avoid bias, the clinical data obtained from the serial study of patients is excluded from the analysis and only the initial observations made in each patient are included. Disease activity in each patient is indicated by a "clinical disease activity" score in which each active disease manifestation is given a score of +1 (Cairns et al 1980).

Clinical disease activity correlated closely with Kc (τ=0.6; $p<0.003$; rank correlation) but not with Kfc (τ=0.11; $p>0.01$; rank correlation) (Fig V.9).

Only five patients had active CNS disease and of these four had low Kc. Two had a previous history of CNS involvement of whom one had normal Kc.

Seven had a history of previous renal involvement and although three had persistent proteinuria (>3gms/24 hours), none had deteriorating renal function or active urinary sediment. Three of these had low Kc.

IMMUNOFLUORESCENCE.

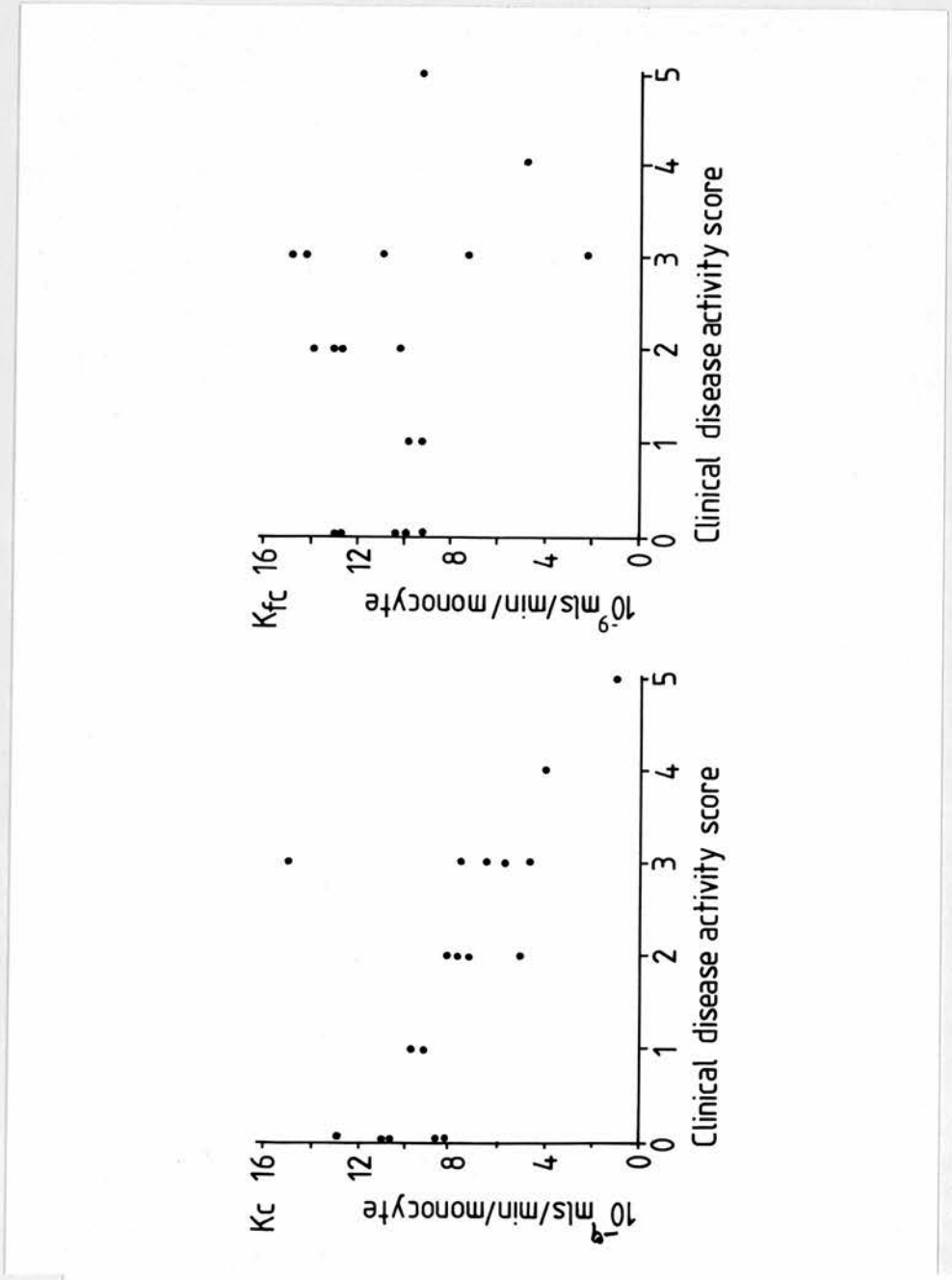
Direct immunofluorescence on monocytes from two patients with low Kfc and low Kc were performed on two separate occasions but no evidence for membrane bound or cytoplasmic associated immune complexes was found.

EFFECT OF DRUGS ON Kc AND Kfc.

No significant association (Fishers two tailed exact test) was found between reduction of Kc or Kfc and treatment with prednisolone, hydroxychloroquine or both in combination.

Fig V.9 Correlation of rates of "complement" receptor (Kc) and Fc receptor (Kfc) mediated phagocytosis with clinical disease activity score.

Clinical disease activity score correlates closely with Kc (τ=0.6;p<0.003;rank correlation) but not with Kfc (τ=0.11).



2.0 EFFECT OF SLE SERUM ON PHAGOCYTOSIS BY NORMAL MONOCYTES.

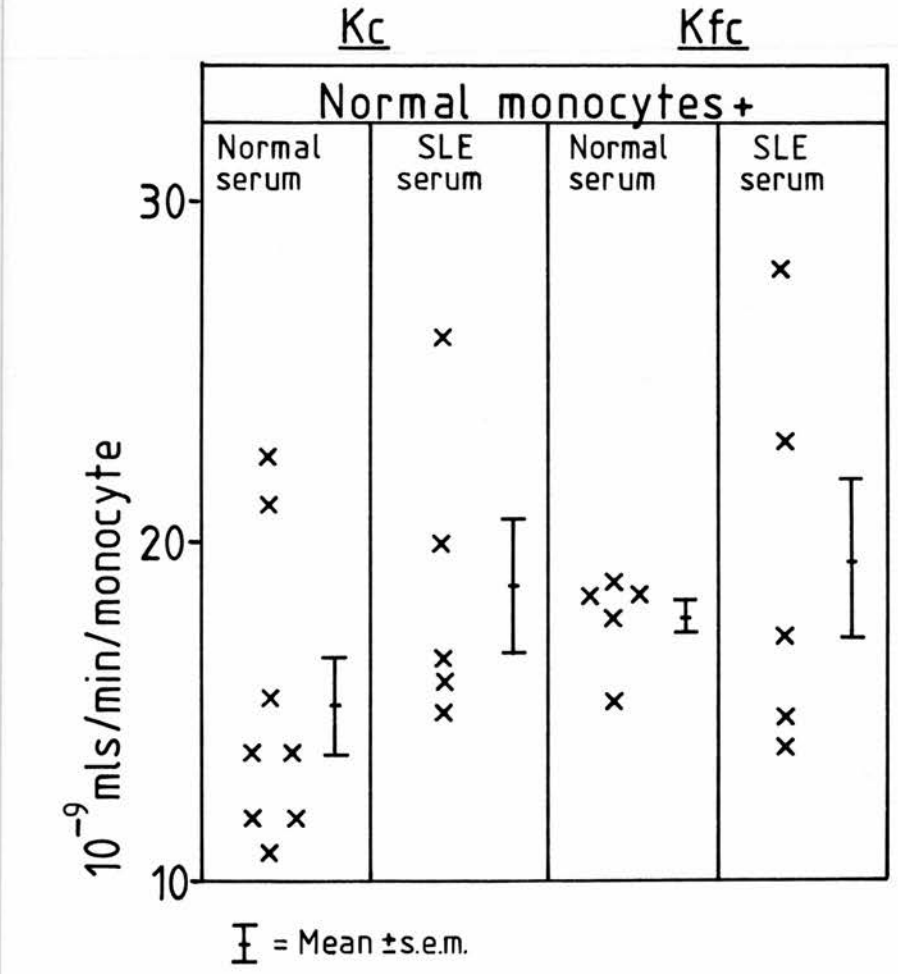
To determine whether serum factors might be responsible for defects of Fc or "complement" receptor mediated phagocytosis, normal monocytes were preincubated with serum from S.L.E. patients with defective phagocytosis. The serum was obtained in each case at the time of drawing blood for each phagocytic assay and was stored in liquid nitrogen until ready for use. Normal control serum was treated in the same way.

Normal monocytes (one donor) were preincubated for 60 minutes at 37°C with either 15% normal control serum, 15% serum from five SLE patients with low Kc or 15% serum from three SLE patients (5 separate serum samples) with low Kfc. Kc and Kfc respectively were then measured in these normal monocytes to determine whether any inhibitory material was present.

No significant inhibition of phagocytosis was detected (Fig V.10) suggesting that immune complexes or other inhibitory factors were not responsible for reduced rates of phagocytosis.

Fig V.10 Effect of SLE serum on phagocytosis by normal monocytes.

Preicubation of normal monocytes with 15% serum from SLE patients with defective monocyte phagocytosis had no significant inhibitory effect, compared with normal serum, on rates of either "complement" receptor (Kc) or Fc receptor (Kfc) mediated phagocytosis (Students t-test).



3.0 RELATION BETWEEN RATES OF "COMPLEMENT" RECEPTOR MEDIATED PHAGOCYTOSIS AND THE CYTOCHEMISTRY OF LARGE MONONUCLEAR CELLS.

Reduced rates of "complement" receptor mediated phagocytosis were not associated with changes in the ratio of NSE positive to large mononuclear cells (>250 cub microns) and even in patients with marked phagocytic defects there was a good correlation between the Coulter "sizing" estimate of numbers of monocytes and the NSE differential.

In only one patient, with a profound reduction in Kc, a discrepancy between the NSE and Coulter "sizing" method of enumerating monocytes was observed. This patient is discussed in further detail below.

4.0 FURTHER STUDIES OF AN SLE PATIENT WITH PROFOUND DEPRESSION OF "COMPLEMENT" RECEPTOR BUT NORMAL Fc RECEPTOR MEDIATED MONOCYTE PHAGOCYTOSIS.

Monocytes from one patient with active SLE (patient C) which exhibited low rates of "complement" receptor mediated phagocytosis were studied in further detail to try and determine more fully the nature of the defect. The aim of these studies was to determine:

i) the effect of therapy on Kc and on the relative numbers of NSE positive and "large" mononuclear cells (>250 cu microns)?

ii) whether a similar defect of "complement" receptor mediated phagocytosis was present in monocytes from other family members which might therefore suggest an intrinsic or genetic defect of "complement" receptor expression?

iii) if it was possible to enhance "complement" receptor expression in this patients cells using an activating agent such as casein?

EFFECT OF CORTICOSTEROID THERAPY ON RATES OF PHAGOCYTOSIS OF "COMPLEMENT" COATED S. CEREVISIAE BY MONOCYTES FROM PATIENT C.

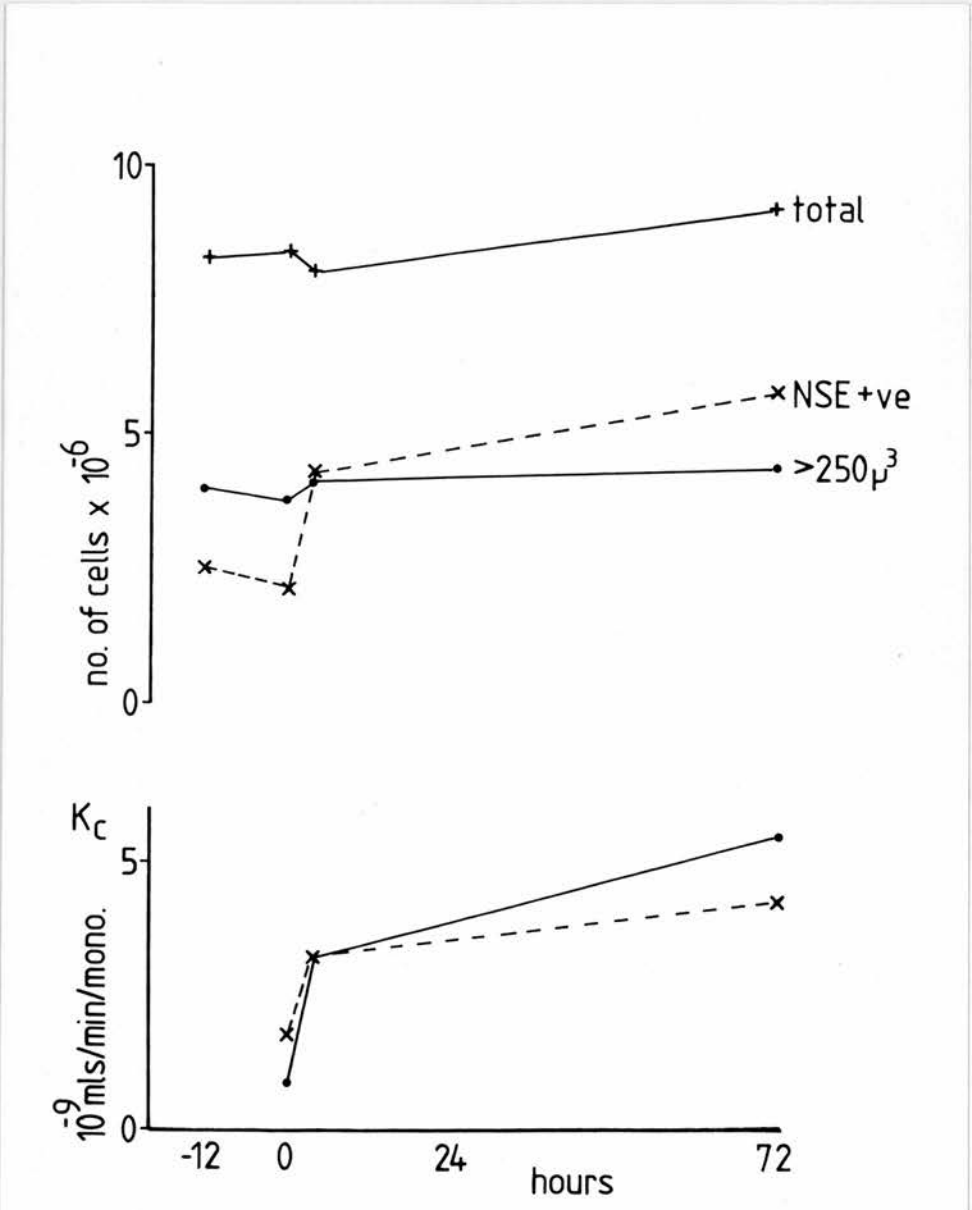
Venous blood was drawn immediately prior to and at 4 and 72 hours after intravenous administration of one gramme of methyl prednisolone. Mononuclear cells were separated in the usual way and numbers of monocytes and lymphocytes determined both by Coulter sizing and by NSE staining. The rate constant Kc was measured as before.

The total yield of mononuclear cells per 20mls of blood rose slightly following steroid administration. However although the percentage of large mononuclear cells (>250cu microns) remained almost constant, the number of NSE positive mononuclear cells doubled during the period of study (Fig V.11). Although Kc remained below normal it rose slightly during the period of study; furthermore the value obtained for Kc was low regardless of whether the monocyte concentration was based on the Coulter or the NSE estimate of monocyte concentrations.

Thus while acute administration of high doses of corticosteroids had a marked effect on relative numbers of NSE positive and negative mononuclear cells and caused a doubling in the absolute number of NSE positive mononuclear cells it did not reverse the defect of "complement" receptor mediated phagocytosis.

Fig V.11 Effect of acute administration of corticosteroids on relative numbers of NSE positive and large (>250 cub micron) mononuclear cells, and on rates of phagocytosis of "complement" coated S. cerevisiae.

72 hours after 1gramme of methyl prednisolone (I.V.) the number of NSE positive mononuclear cells has doubled while the number of large (>250 cub microns) cells is almost unchanged.
A rise in Kc is seen, regardless of whether the monocyte concentration is calculated from the %age of NSE positive cells (- - - -), or the %age of large cells (———). However the highest value of Kc is still well below the normal range (normal > 7.9×10^{-9} mls/min/monocyte).



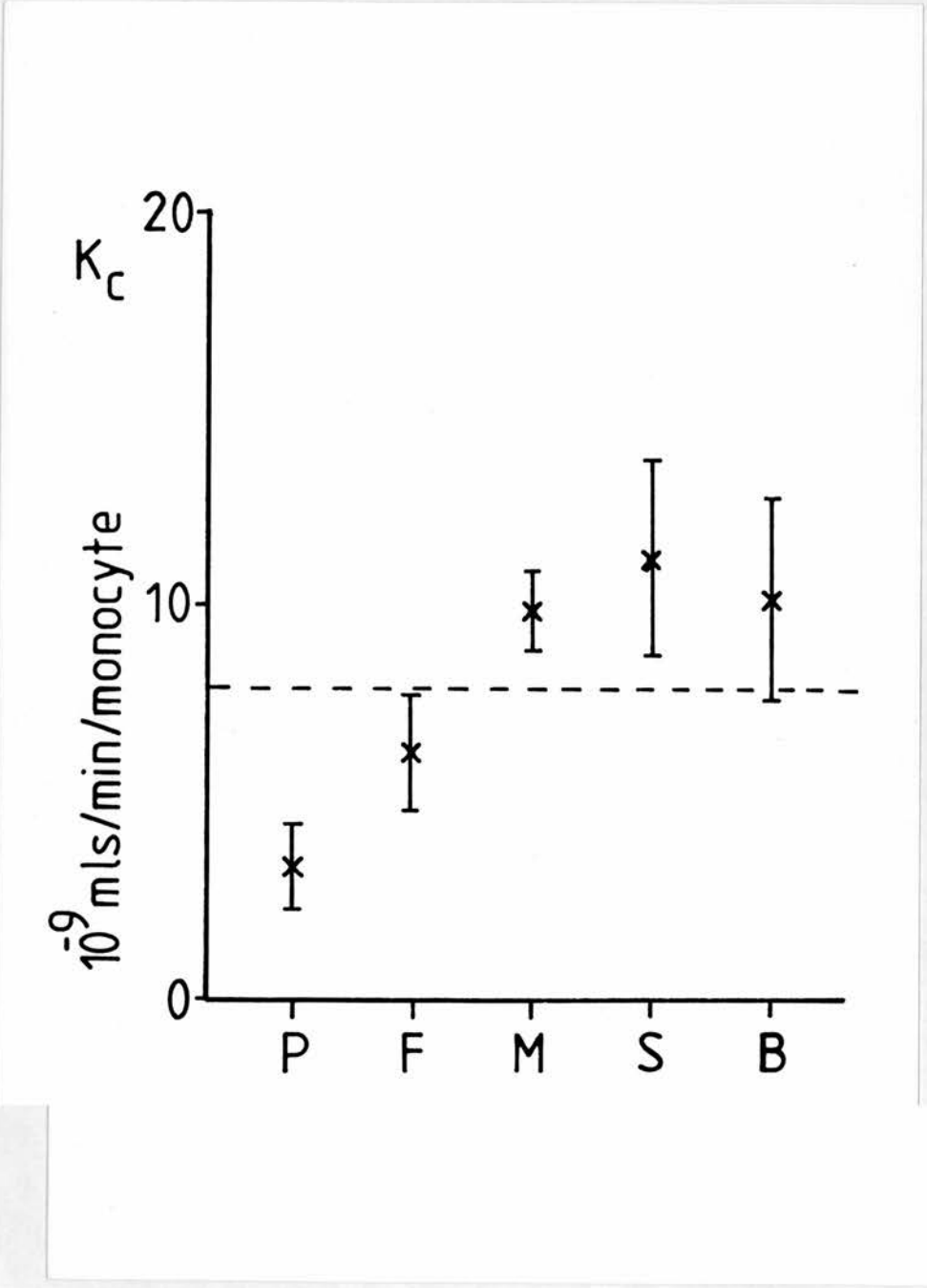
FAMILY STUDY OF "COMPLEMENT" RECEPTOR MEDIATED MONOCYTE PHAGOCYTOSIS.

The relatives of patient C (father, mother, sister and brother) were studied to seek evidence for a genetically determined defect of monocyte "complement" receptor phagocytic function. The relatives were in good health apart from the mother who had long standing discoid lupus erythematosus. Mononuclear cells were obtained from each relative on three separate occasions for determination of Kc which was measured using "complement" coated S. cerevisiae as before.

Monocytes from the mother, brother and sister gave values for Kc which were no different from the normal controls whereas Kc for monocytes from the father lay below the normal control range (Fig V.12).

Fig V.12 Mean (+/-S.E.M.) of three determinations of Kc in monocytes from patient C and her relatives.

Monocytes from the patient (P) and her father (F) have low mean Kc, while monocytes from the mother (M), sister (S) and brother (B) appear to be normal.



ENHANCEMENT OF "COMPLEMENT" RECEPTOR MEDIATED PHAGOCYTOSIS

To test the possibility that defective "complement" receptor mediated phagocytosis by SLE monocytes was due to excessive "masking" of complement receptors or failure of monocyte activation attempts were made to enhance phagocytosis of "complement" coated yeast using casein as an in vitro activating agent.

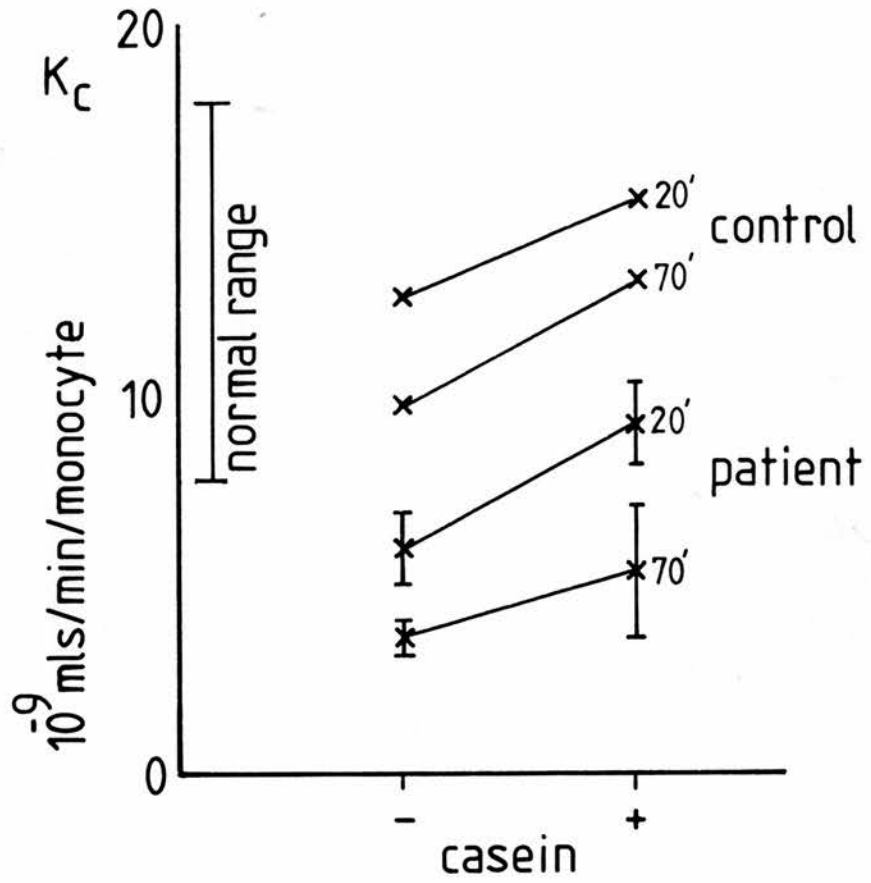
In each experiment 150ul of cell suspension were incubated either with 20ul of casein solution (3.0mg/ml final) in HBSS or with 20ul of HBSS alone. Preincubation was performed for either 20 or 70 minutes. Control experiments were carried out on normal mononuclear cells for comparison.

The results are shown in Fig V.13. Preincubation of either normal or patients' cells with HBSS alone for 70 minutes resulted in lower values of Kc than preincubation for 20 minutes. However in the presence of casein both control and patients cells showed an increase in phagocytosis of "complement" coated yeast (Kc). The mean Kc for the patients' cells rose by 55% and 46% after 20 and 70 minutes preincubation and for the normal control cells by 20% and 34% respectively.

Thus casein enhanced "complement" receptor activity both in monocytes from patient C and in normal monocytes in the expected fashion confirming the presence of cryptic receptors. However the increase in SLE monocytes paralleled that seen in normal monocytes and it therefore seems unlikely that there is an excess of cryptic receptors which are not fully expressed on monocytes from SLE patients.

Fig V.13 Effect of casein on rates of monocyte phagocytosis of "complement" coated yeast (Kc).

20 or 70 minutes preincubation with casein stimulates the mean rate of phagocytosis of "complement" coated yeast by both SLE (n=3) (+/-S.E.M.) and normal monocytes (n=2) to a similar extent.



5.0 PHAGOCYTOSIS OF IgG AND "COMPLEMENT" COATED YEAST BY MONOCYTES FROM NORMAL SUBJECTS WITH THE HLA-B8 HAPLOTYPE

A recent study has demonstrated impaired in vivo "Fc-mediated" clearance of IgG coated erythrocytes in apparently healthy individuals with the HLA-B8/DRW3 haplotype (Lawley et al 1981) and the authors suggest that this may reflect an intrinsic defect of MPS function which could predispose to immune complex disease. However others have not found any impairment of Fc receptor expression in blood monocytes from such individuals (Fries et al 1982).

To determine whether any functional impairment of Fc or "complement" receptor mediated phagcoytosis could be detected in HLA-B8 positive subjects, which might provide a genetic basis for the abnormalities found in the SLE patients described above, rates of phagocytosis by monocytes from a group of HLA-B8 normal subjects were measured.

Eleven healthy HLA-B8 positive medical staff (6 male; 5 female) mean age 24 years (range 21-26) were studied. These individuals were kindly provided from a register of tissue-typed individuals by Dr M. Steele (MRC Unit; Western General Hopsital, Edinburgh). Their full HLA-A & B haplotype is shown in Table 6.13.

Table 6.13 HLA-A & B tissue type of panel of healthy B8 subjects.

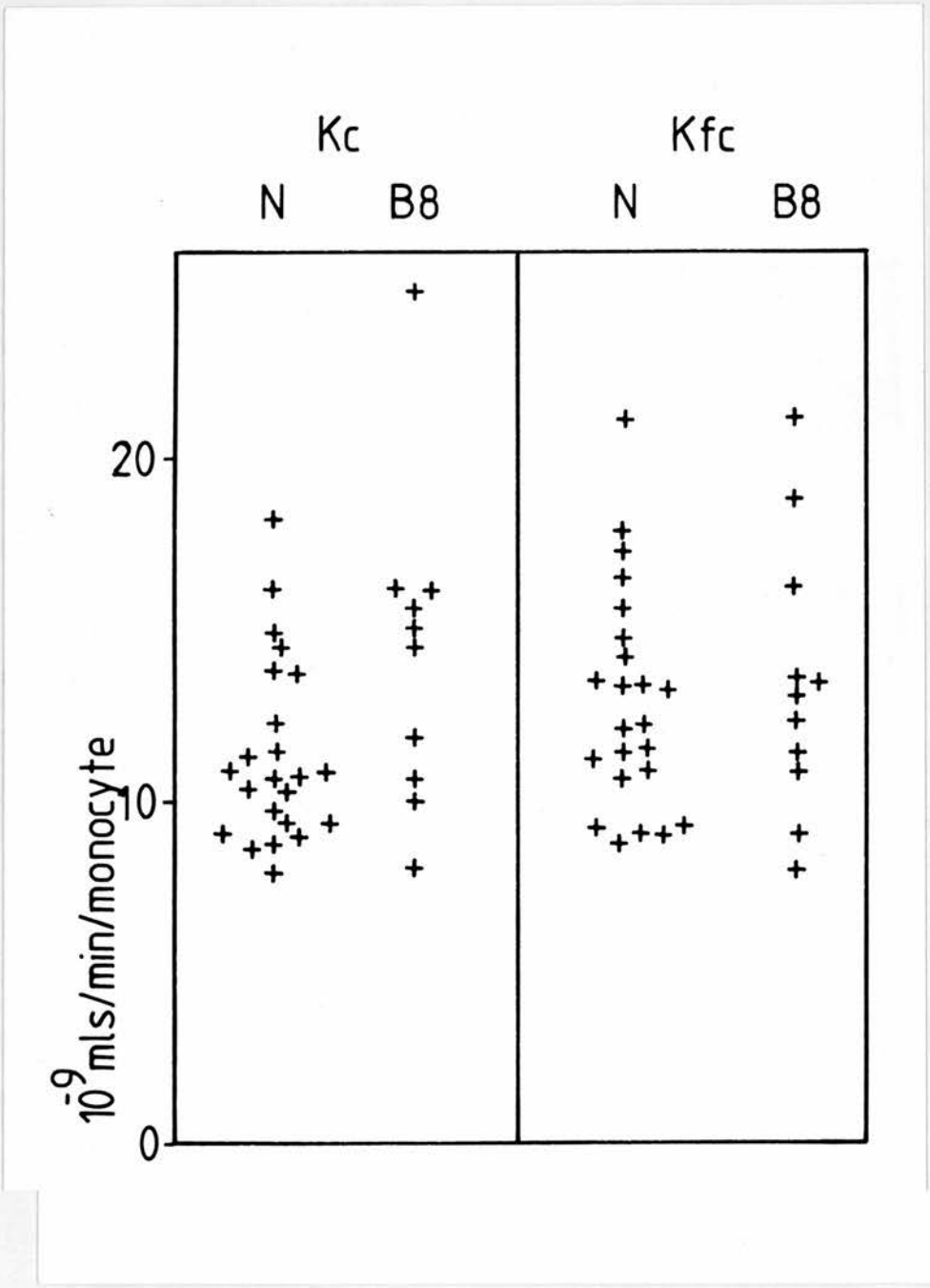
Patient	HLA-A	HLA-B
1	10/11	8/W15
2	2/10	8/W22
3	1/10	7/8
4	1/2	8/W16
5	1/11	8/18
6	1/11	8/18
7	1/?	8/?
8	1/2	8/12
9	1/11	7/8
10	1/2	8/?
11	1/3	7/8

Fc AND "COMPLEMENT" RCEPTOR MEDIATED PHAGOCYTOSIS BY MONOCYTES FROM HLA-B8 POSITIVE NORMAL SUBJECTS

There was no significant difference (Fishers exact test) in the rate constants for "complement" receptor or Fc receptor mediated monocyte phagocytosis (Kc and Kfc) between the normal control group and HLA-B8 positive individuals (Fig V.14).

Fig V.14 Rate constants for "complement" receptor (Kc) and Fc receptor (Kfc) mediated monocyte phagocytosis in normal controls and HLA-B8 subjects.

There is no significant difference between the normal controls and the HLA-B8 subjects (Fishers exact test).



6.0 COMPOSITION OF THE MONONUCLEAR CELL POPULATION IN NORMAL CONTROLS AND SLE PATIENTS

A limited number of preliminary experiments have been performed on mononuclear cells from SLE patients to determine whether reduced rates of "complement" receptor mediated phagocytosis reflect a reduction in the number of CR bearing monocytes as demonstrated above in RA patients. Only 6 SLE patients have so far been studied, five of whom were in complete remission and one had active disease. The method of assessing percentages of NSE positive and percentages of phagocytic cells is exactly as described above (p290) for RA patients. The same group of 11 normal control subjects has been used (p288).

SLE PATIENTS:

The serological features of these eight SLE patients (7 female, 1 male) (ARA criteria) are shown in Table 6.14 and drug therapy in Table 6.15.

Table 6.14 Clinical and serological features of SLE patients

Patient number	Age yrs	Hb gm/dl	WBC 10/l	ESR mm/1st hr.	Clinical score
1	16	10.5	2.7	101	6 *
2	27	13.3	3.6	7	1
3	34	13.1	10.5	-	0
4	50	14.5	6.5	4	0
5	22	13.8	3.2	1	1
6	26	11.3	4.1	62	1
7	17	12.2	3.3	25	1
8	31	12.1	4.4	30	1

Patient number	ANF	DNA u/dl	C3 mg/dl	C4 mg/dl	ENA
1	+	115	40	10	Sm/RNP
2	+	<25	60	13	Ro/La
3	-	<25	67	24	Sm
4	+	<25	63	16	-
5	+	<25	63	29	-
6	+		64	28	Sm/RNP
7	+		60	17	Sm/RNP
8	+		64	8	Sm

(* arthritis; leucopenia; PUO; glomerulonephritis; malar rash; vasculitis; splenomegaly/lymphadenopathy)

Table 6.15 Drug therapy of SLE patients

Patient number	Therapy A mg/day	B mg/day	D
1	-	-	+
2	-	200	-
3	8	-	-
4	-	400	-
5	-	-	-
6	12	400	+
7	15	400	+
8	-	-	+

A = prednisolone;
B = hydroxychloroquine;
C = NSAID.

RESULTS

The preliminary results, which are shown in Table 6.16, suggest that a much smaller percentage of monocytes from the patient with active SLE (clinical score = 6) are capable of phagocytosing "complement" coated yeast than IgG coated yeast, but no difference is seen in mononuclear cells from the seven patients with clinically inactive SLE (clinical score = 0 or 1).

There also appears to be a reduction in the number of NSE positive cells capable of phagocytosing either type of particle in the SLE group compared with the normal controls. Whereas 80% of NSE-positive cells from normal controls are phagocytic, only 60% of NSE positive mononuclear cells from SLE patients are phagocytic for either particle. However it must be emphasised that the numbers are too small to draw firm conclusions.

Table 6.16 Percentage (+/-S.D.) of cells in mixed mononuclear cell population phagocytosing IgG coated yeast (%FcR positive) or "complement" coated yeast (%CR positive) and percentage (+/-S.D.) NSE positive cells.

	Mononuclear cells			
	n	% NSE +ve	% FcR +ve	% CR +ve
Normal controls	11	27.0 +/-6.2	21.1 +/-3.7	21.1 +/-4.0
"Inactive" SLE	7	31.9 +/-2.4	18.2 +/-6.8	18.6 +/-5.9
"Active" SLE	1	25.8	17.8	7.4

CHAPTER 7

DISCUSSION

DISCUSSION

Monocyte separation

To avoid selection or loss of subpopulations of monocytes with abnormal properties, mixed mononuclear cell preparations were used during phagocytic studies and no attempt was made to separate monocytes from lymphocytes on the basis of functional properties (e.g. adherence) or physical properties (e.g. density). Although there is the possibility that the presence of lymphocytes might influence phagocytic function, it was felt that this was unlikely to be critical during short term studies lasting up to an hour.

However, distinction of monocytes from lymphocytes was of considerable importance in quantitative studies where the monocyte concentration formed an integral component of rate calculations. Two methods were used to estimate monocyte concentrations - a Coulter sizing method and NSE staining. The Coulter sizing technique, which identifies monocytes by their larger volume, counts large numbers of cells rapidly and reproducibly. However it has the disadvantage that there is overlap in the size distribution curves of monocytes and lymphocytes and changes in numbers of the large lymphocyte population or an increase in numbers of smaller monocytes will give misleading results. Diffuse NSE staining is widely accepted as a marker of mononuclear phagocytes and was used as an additional criterion of relative numbers of lymphocytes and monocytes. The principal disadvantages are that it is more time

consuming and, while tissue macrophages stain strongly and uniformly, blood monocytes vary considerably in the intensity of staining and may give rise to uncertainties in identification. The use of two markers - the first using large numbers of cells and a physical characteristic and the second using smaller numbers of cells but a more specific cytochemical marker, was thus a suitable compromise.

Phagocytic assay system

Two methods for measuring phagocytosis by blood monocytes were used in these studies. The first required direct microscopic identification of phagocytic cells and the second measured rates of phagocytosis indirectly by monitoring the fall in extracellular yeast concentration in a mixture of monocytes and yeast.

An initial pilot study of a direct microscopic method of assessing phagocytosis described by Territo and Cline (1977) gave disappointing results but was later used successfully in a modified form to identify phagocytic subpopulations. In the original method yeast were used as substrate and phagocytosis allowed to proceed in the presence of 10% AB serum; phagocytosis was assessed by counting numbers of phagocytic cells and ingested yeast in cytocentrifuge slides of cells stained with Jenner-Giemsa. During the assay large clumps of yeasts and monocytes formed making subsequent microscopic counting of cells and yeast difficult. Furthermore, monocytes were also rapidly lost from suspension as a result of adherence to the wall of the incubation vessel. The latter problem was overcome by

incubating cells in wells machined from Teflon block. Clumping of cells and yeast did not occur providing preopsonised yeast were used. The "direct" method employing yeast preopsonised with different opsonins was later successfully applied to the identification of subpopulations of phagocytic cells. Also, since Jenner-Giemsa was found to be an unsatisfactory stain for these studies, NSE stains were used which enabled clearer distinction of phagocytic and nonphagocytic monocytes from lymphocytes.

For studies of the kinetics of phagocytosis and subsequent clinical studies, an "indirect" assay described by Leijh et al (1977) was employed. The method monitors the rate of phagocytosis by following the fall in extracellular yeast concentration in a mixture of yeasts and monocytes and no attempt is made to count numbers of ingested yeast.

The method was validated by metabolic controls studies. N-ethyl maleimide which irreversibly inhibits thiol dependent enzyme systems including anaerobic glycolysis caused dose dependent inhibition of phagocytosis while cyanide, an inhibitor of mitochondrial respiration had no effect. Since N-ethyl maleimide is relatively nonspecific and may for example inhibit Fc receptor binding, the effect of 2-deoxyglucose (2DG) which is a more specific inhibitor of anaerobic glycolysis was also studied. However, in agreement with Michl (1976), 2DG caused only partial inhibition of phagocytosis. The failure of 2DG to produce complete inhibition has been attributed to the presence of stores of creatine phosphate which is used by macrophages to rephosphorylate

ADP to ATP and provide energy for phagocytosis even when anaerobic glycolysis is inhibited. Creatine phosphate stores are created by phosphorylation of creatine by ATP in resting cells, thus to produce complete metabolic inhibition of phagocytosis, simultaneous inhibition of anaerobic glycolysis by 2DG and depletion of ATP dependent synthesis of creatine phosphate in resting cells is required. This was achieved by preincubating cells with 2DG and cyanide simultaneously and under these conditions 100% inhibition of phagocytosis was obtained. Qualitative evidence that the "indirect" method was a measure of phagocytosis was obtained by electron microscopy.

Nature of opsonins present on yeast

As the studies progressed, the nature of the opsonins present on the phagocytic particle assumed greater importance and control studies were performed to identify the type of opsonins present.

In initial studies, C. albicans opsonised with human serum was used which was found on direct immunofluorescence to be coated with IgA, IgM, IgG and C3/C3c (β1c/β1a). Thus Fc receptors, CR1 and CR3 receptors and possibly others were potentially involved in mediating binding and ingestion of this particle. The involvement of lectin-like receptors was also considered, and although unopsonised particles were ingested very slowly, saccharide ligands may none the less act synergistically with opsonins such as complement in mediating ingestion. Using serum opsonised C. albicans, reduced rates of phagocytosis were found in monocytes

from certain RA patients and as a first step towards resolving which type of receptor was involved in the abnormality, two different types of preopsonised yeast were developed. To study Fc receptor mediated uptake, C. albicans opsonised with pooled human IgG was used and for "complement" receptor mediated uptake, S. cerevisiae preopsonised with serum from a single donor was used.

To examine which ligands were responsible for mediating phagocytosis these two types of particle were treated with F(ab')2 fragments of IgG antibody to potential ligands including Fc, Clq, C4, βlH, C3/C3c (β1c/β1a). Anti C3/C3c does not enable a distinction to be made between C3b and C3bi. Treatment of the IgG coated C. albicans with F(ab')2 anti-Fc produced complete inhibition of phagocytosis confirming that uptake was mediated by Fc receptors. However it is not known whether IgG1 or IgG3 is the more important ligand. Phagocytosis of "complement" coated S. cerevisiae was not inhibited by F(ab')2 anti-C4 or βlH but was strongly inhibited by anti-C3/C3c and, unexpectedly, to a small extent by anti-Clq. Simultaneous treatment of these yeast with anti-C3 and anti-Clq produced complete inhibition of ingestion. Although it has been reported that monocytes carry a receptor for Clq there have been no reports to suggest that these receptors are involved in phagocytosis. However the contribution of Clq appeared to be small and binding and ingestion was mainly dependent on C3/C3c and therefore probably involves either the CR1 or CR3 receptors. Further studies using anti-CR1 or anti-CR3 antibody as inhibitors are required to clarify this issue.

Kinetics of phagocytosis

Using the "indirect" method the kinetics of monocyte phagocytosis of yeast opsonised in various ways were studied. The results of pilot studies of monocytes from normal controls and RA patients demonstrated that phagocytosis followed simple second order collision kinetics and enabled a "rate constant" to be obtained from a rapid 20 minute study. The "rate constant" is a measure of the efficiency of binding and phagocytosis and, as demonstrated, is dependent on the degree of opsonisation of the yeast particles. A major advantage of calculating a "rate constant" is that it incorporates the monocyte concentration and avoids the need for accurate adjustment of the cells to a specific concentration in each experiment.

In later studies where the aim was to determine the maximum number of monocytes capable of phagocytosing either IgG or "complement" coated yeast, cells were incubated with yeast for 40 minutes and allowed to reach the plateau phase of the phagocytic rate curve.

Results of studies on monocytes from RA patients

In general, identification of monocytes by Coulter sizing correlated well with NSE staining of mononuclear cells from RA patients and the Coulter sizing result was used for calculation of the phagocytic rate constant. However as will be discussed below, the Coulter sizing methoddid not always correlate with NSE staining, giving rise to problems in the interpretation of kinetic data.

Initial studies of monocytes from RA patients using C. albicans opsonised with serum demonstrated a significant reduction in rates of phagocytosis (Ks) in patients with rheumatoid cutaneous vasculitis (RV) but not in patients with uncomplicated RA. Monocytes from one of the RV patients were restudied after cessation of the vasculitic episode and found to have normal rates of phagocytosis, suggesting that the abnormality was transient and secondary to the disease process.

Cutaneous vasculitis in RA is associated with hypocomplementaemia (Mongan et al 1969) and large circulating 19S IgM containing immune complexes (Weisman & Zvaifler 1975) and as expected there was a tendency for the RV patients to have reduced levels of serum haemolytic complement. This suggested that complement fixing circulating immune complexes might be responsible for the phagocytic abnormality, perhaps by causing blockade of monocyte receptors. However immunofluorescence studies on monocytes from three of the RV patients revealed no intracytoplasmic

inclusions of gamma-globulin or complement thus providing some evidence against this hypothesis.

It was also not clear from this preliminary study which types of monocyte receptor were involved in the functional abnormality of phagocytosis and to examine this question further the studies were repeated using IgG coated C. albicans to measure rates of Fc receptor mediated phagocytosis, and serum opsonised S. cerevisiae to measure "complement" receptor mediated uptake. The results were correlated with tests for immune complexes and serum complement levels.

The results of this second study demonstrated that reduced rates of phagocytosis by monocytes from patients with cutaneous vasculitis were confined to uptake of "complement" coated yeast and that phagocytosis of IgG coated yeast was normal. In addition, four patients who did not have vasculitis also had a similar phagocytic defect. Two of these four had pyoderma gangrenosum, a known association of both RA and seronegative arthritis (Holt et al 1980), one had nodular RA in association with primary biliary cirrhosis (James et al 1981) and the fourth had nodular RA complicated by pericarditis. None of the remaining patients, who included two with pyogenic infection and one with Feltys syndrome, had a phagocytic defect.

The phagocytic data was examined for correlations with circulating immune complexes and serum complement. Hypocomplementaemia, elevated anti-complementary activity (ACA) and Clq binding activity (Clqba) were features of many of the patients

with reduced "complement" receptor mediated phagocytosis (Kc). However, reduced Kc did not always correlate with these serological changes and of four patients with reduced Kc without vasculitis, two had normal C3 and C4 levels and three had no increase in ACA. Conversely, normal Kc was seen in some patients with very high levels of Clqba, lowered serum C3 and C4 levels and elevated ACA titres. Thus the association between serological evidence of complement fixing circulating immune complexes and reduced Kc was not invariable. Although this evidence suggests that abnormalities of "complement" receptor mediated phagocytosis may not be due to receptor blockade by immune complexes, it is by no means conclusive. For example the finding of normal levels of immunoreactive C3 does not preclude complement consumption since increased complement synthesis may be occurring simultaneously. Conversely, the failure to demonstrate abnormalities of phagocytosis in all patients with evidence of circulating immune complexes may simply reflect a lack of sensitivity in the phagocytic assay system. None the less, direct immunofluorescence on mononuclear cells from patients with reduced Kc again failed to reveal the presence of monocyte associated immunoglobulin or complement, suggesting that receptor blockade by immune complexes was not responsible for the functional alteration of phagocytosis. Furthermore, incubation of normal monocytes with serum from RA patients with reduced Kc did not inhibit their ability to phagocytose "complement" coated yeast.

An alternative hypothesis, namely that reduced Kc was due to

an alteration in the composition of the circulating pool of blood monocytes with a concomitant reduction in function was therefore considered. This could arise either by dilution of the peripheral blood pool of monocytes with immature monocytes prematurely released from the marrow or alternatively by increased removal of the most functionally active cells from the blood stream or by a combination of both these mechanisms. The net effect of either mechanism might be to reduce the mean functional "complement" receptor activity of circulating monocytes accessible to sampling. Furthermore if increased removal and margination of monocytes were secondary to phagocytosis of immune complexes within the circlation, this would explain the failure to demonstrate monocyte associated immune complexes as the relevant cells would no longer be accessible for study.

In support of an "altered population" hypothesis was the finding that in some patients there was a discrepancy between the Coulter and NSE estimates of monocyte concentrations and that reduced Kc correlated with the magnitude of this discrepancy. Thus the data appeared to show that reduced Kc correlated with the appearance of increasing numbers of NSE-negative, large (>250 cub microns) mononuclear cells. Since Kfc remained normal, the data implied that there were large NSE-negative cells with phagocytic Fc receptors but functionally inactive or absent "complement" receptors appearing in the circulation. However because cytochemical data was not available on every patient there was the possibility of bias, and further studies of patients with defective "complement" receptor mediated phagocytosis were undertaken. These studies sought to confirm or refute the hypothesis that changes in

phagocytic function reflected an alteration in monocyte subpopulations and the appearance of NSE-negative, FcR-positive, CR-negative mononuclear phagocytes. In these experiments the percentages of mononuclear cells showing either Fc or "complement" receptor dependent phagocytosis (FcR and CR positive cells) were enumerated and compared with the percentage of NSE positive mononuclear cells and the percentage of large (>250 cub microns) mononuclear cells. Mononuclear cells were obtained from normal controls, patients with uncomplicated RA, and RA patients with vasculitis or a history of vasculitis. The results confirmed that there is a reduction in the percentage of monocytes capable of phagocytosing "complement" coated yeast and no reduction in the percentage of FcR positive cells. The results did not confirm that there was an increase in numbers of NSE-negative large mononuclear cells bearing phagocytic Fc receptors. The data therefore gives only partial support to the previous findings and demonstrates the presence of NSE-positive, FcR positive, CR negative cells. Although the presence of NSE-negative phagocytic cells was not confirmed, the possibility remains that the CR deficient cells are immature monocytes prematurely released from the marrow.

Horwitz and Steagall (1972) reported increased numbers of non-phagocytic, NSE negative monocyte precursors in the peripheral blood of patients with RA. They used latex particles to measure phagocytic function and Fc and "complement" receptor function was not measured directly. Thus a direct comparison with the data

reported in this thesis is not possible. More recent studies of the functional and cytochemical characteristics of monocyte precursors have produced conflicting data. Lohmann-Mattes et al (1979) have identified a murine bone marrow derived monocyte precursor which is non-adherent, nonphagocytic and NSE negative and has natural killer cell (NK) activity. They have shown that this NSE negative cell matures into a typical NSE positive mononuclear phagocyte on culture in vitro. These observations have been extended to human peripheral blood where similar nonadherent, NSE-negative mononuclear cells with NK activity have been identified which mature into NSE-positive macrophages (Lohmann-Mattes et al 1981). The phagocytic function of this cell has not been reported. On the other hand, studies of human bone marrow cells (van Furth et al 1980), identified the human promonocyte as a large NSE positive cell with a phagocytic Fc receptor. These cells carry C3b receptors which have little if any phagocytic activity. The cytochemical and functional properties of human monoblasts have not been fully characterised. Studies of murine marrow precursors (van Furth et al 1980) show that only 30% of promonocytes are NSE positive and are less phagocytic than their human counterparts.

Although the "CR-negative" cells identified in these experiments appear to have the characteristics of promonocytes i.e. they are NSE-positive and have a phagocytic Fc receptor (van Furth et al 1980), the results do not preclude the possibility that the inability to phagocytose "complement" coated yeast might be due to the effect of serum factors such as immune complexes.

A prequisite for further characterisation of these cells is their isolation from other mononuclear cells. Preliminary experiments, described in this thesis, have been performed on monocytes from a limited number of subjects in which the phagocytic function of the glass adherent population has been compared with that of the whole mononuclear cell population in suspension. These experiments suggest that the CR-negative monocytes are not glass-adherent and in this respect resemble the monocyte precursors described by Lohmann-Mattes et al (1981). If further experiments confirm that these cells are non-adherent, this property could provide a basis for their isolation and further study.

Conclusions

The aim of these studies was to determine whether defects of phagocytosis by mononuclear phagocytes are present in RA patients which might contribute to the persistence of immune complexes.

The experimental results show that functional defects of "complement" receptor mediated phagocytosis are found in blood monocytes from RA patients with vasculitis and certain other "extra-articular" complications. However, the abnormalities when present are transient and appear to be secondary to the disease process. The evidence suggests that alterations in phagocytic function reflect alterations in the composition of the monocyte population which may be secondary to release of monocyte precursors from the bone marrow. However, the possibility that immune complexes are responsible for alterations in phagocytic function

either directly by "receptor blockade" or indirectly by causing increased emigration of monocytes from the blood cannot be completely excluded on the available evidence.

The importance of using more than one criterion for identifying monocytes is emphasised by the findings in these studies as well as the results of studies of monocyte precursors reported by others (Lohmann-Mattes et al 1979, 1981).

Results of studies on monocytes from SLE patients

The initial studies of a group of 18 SLE patients demonstrated a reduction in rates of phagocytosis of both IgG and complement coated yeast by blood monocytes from some patients with SLE. Reduced uptake of IgG coated yeast was an infrequent finding, but was correlated with reduced serum C3 levels and, in two patients studied serially, with disease activity. In the group as a whole however, there was no correlation between Kfc and the "disease activity" score. Reduced phagocytosis of "complement" coated yeast on the other hand was found in monocytes from 50% of SLE patients, correlated well with "disease activity" but not with any of the serological parameters measured. It should be noted however that in one patient studied longitudinally, phagocytosis of "complement" coated yeast tended to be depressed regardless of disease activity suggesting that the functional abnormality might not be a secondary phenomenon.

The data suggests that different factors may be responsible for altering Fc and "complement" mediated phagocytosis. Several mechanisms might be responsible and include blockade of phagocytic receptors by circulating immune complexes; the presence of monocyte subpopulations with altered phagocytic receptor function; modulation of phagocytosis by drugs; and intrinsic abnormalities of monocyte receptor expression.

Although no correlation was found between reduced Kc and reduced serum complement levels or tests for immune complexes, a

positive correlation was noted between KFc and serum C3 levels which could be the result of immune complex blockade by Fc bearing, complement fixing immune complexes. However, direct examination with immunofluorescence failed to reveal the presence of membrane bound or cytoplasmic immune complexes. Furthermore, serum from SLE patients with marked defects of phagocytosis had no inhibitory effect on phagocytosis by normal monocytes, a finding which is agreement with Svensson et al (1980).

Dilution of the peripheral blood monocyte pool with immature cells could provide an alternative explanation for a reduction in "complement" receptor mediated monocyte phagocytosis. It should be noted that with the exception of one patient (patient C) discussed below, there was a good correlation between the Coulter sizing and NSE staining methods for estimating monocyte concentrations. Thus the discrepency between these two methods of assessing monocyte concentrations noted during studies of RA patients did not occur in studies of SLE patients. As discussed in relation to RA patients the premature release of immature cells from the marrow in response to an inflammatory stimulus could dilute the blood monocyte population and result in reduction of numbers of monocytes expressing functional "complement" receptors. The correlation between reduction in Kc and increasing disease activity in SLE patients supports this possibility. This hypothesis was tested further by measuring the relative percentages of mononuclear cells which were capable of phagocytosing either IgG or "complement" coated yeast particles. The results of these preliminary studies

identified one patient with active SLE with a marked reduction in the percentage of monocytes phagocytosing "complement" coated yeast while the remaining patients with mild or inactive disease had normal percentages of phagocytic cells. This preliminary data suggests that there may be a reduction in the proportion of monocytes capable of phagocytosing "complement" coated yeast in active SLE, and although studies of more patients are clearly required, the results are very similar to those obtained in RA patients with vasculitis.

No inhibitory effect of drug therapy on monocyte function was found in the patients considered as a group or in two patients (A&B) studied serially. In one patient (C) with depressed phagocytosis of "complement" coated yeast an increase in rates of phagocytosis was seen up to 72 hours after a single intravenous dose of 1gm of methylprednisolone. An enhancing effect of steroid therapy on phagocytosis of serum opsonised cryptococci by monocytes has been reported previously (Rinehart et al 1975). The fact that the number of NSE positive cells in the mononuclear cell population obtained from this patient doubled during the period of study while the the number of large (>250 cub microns) cells remained constant strongly suggests that steroids are influencing monocyte traffic and that increased numbers of smaller NSE positive monocytes were present in the circulation. The associated increase in "complement" receptor dependent phagocytosis suggests that these may be relatively mature cells derived from the marginated pool of monocytes which are about to leave the circulation for tissue

sites.

The possibility of intrinsic abnormalities of mononuclear phagocyte receptors analogous to the genetic defect of low density lipoprotein receptors found in familial hypercholesterolaemia (Goldstein et al 1975) should also be considered. Although one study (Lawley et al 1981) has demonstrated abnormal in vivo Fc mediated RES clearance in 50% of healthy B8/DRW3 positive subjects, a more recent study (Fries et al 1982) of the number and affinity of blood monocyte Fc receptors in B8 positive individuals showed no abnormality. Similarly no Fc or "complement" receptor dependent phagocytic defect was found in monocytes from a group of eleven B8 healthy subjects described in this thesis. The significance of studies suggesting that there may be genetic variation in erythrocyte C3b receptor (CR1) density and that low densities of CR1 are found more frequently in patients with SLE and some of their healthy relatives (Miyakawa et al 1982; Wilson et al 1982) has already been discussed in Chapter 3. To test the hypothesis that defective "complement" receptor dependent phagocytosis by monocytes might be inherited, monocyte phagocytic function was studied in the relatives of one SLE patient (patient C) with reduced Kc. Monocytes from the father of this patient showed reduced rates of phagocytosis, while monocytes from 2 siblings and the mother were normal. Although the result is consistent with the presence of an inherited defect of "complement" receptor phagocytic function, the possibility remains that "complement" receptor

deficiency has nothing to do with the occurrence of SLE in this patient. Studies of healthy control families as well as of "SLE families" will be required to answer this question. In contrast, other studies suggest that reduced erythrocyte CR1 expression in SLE patients (Iida et al 1982) and absent glomerular CR1 in SLE patients with diffuse proliferative nephritis (Kazatchkine et al 1982) is likely to be secondary to the disease process and the presence of intrinsic complement receptor defects is by no means certain.

To test the possibility that "masking" of receptors was responsible for defective "complement" receptor mediated phagocytosis, the effect of casein was examined on monocytes from an SLE patient (patient C). Although casein enhanced phagocytosis of "complement" coated yeast by monocyte from this patient, confirming the presence of latent receptor activity, the increase was no greater than that seen in normal controls suggesting that there was no excess of cryptic receptors in the defective monocytes.

Conclusions

The aim of these studies was to determine whether defects of phagocytosis by mononuclear phagocytes are present in SLE patients which might contribute to the persistence of immune complexes. The studies have identified functional defects of both Fc and "complement" receptor mediated monocyte phagocytosis and the possible significance of these findings has been discussed.

Some of the evidence suggests that defective "complement" receptor mediated phagocytosis may be secondary to disease activity while data from a family study suggests that it may be inherited. Abnormalities of Fc receptor mediated phagocytosis appeared to be infrequent and when present were related to changes in disease activity. The exact nature of the defect of "complement" receptor mediated phagocytosis remains to be established and may involve CR1, CR3 or possibly C1q receptors.

Whatever their cause these functional abnormalities of monocyte phagocytosis have important implications. Initial contact with antigen involves opsonisation by antibody and complement and is followed by phagocytosis. The development of an effective immune response is dependent on this phagocytic step as well as on subsequent presentation of antigen to lymphoid cells. Inefficient phagocytosis of antigen could therefore impair the subsequent immune response, allow persistence of antigen and favour the development of immune complex disease. The same phagocytic defect could also contribute to the persistence of immune complexes and thus exacerbate the immune defect.

Suggestions for further experiments

The significance of diminished "complement" receptor function in blood monocytes and its relation to the pathogenesis of RA and SLE must remain uncertain until the mechanism of the alteration is understood. Additional experiments which might help to further elucidate the abnormality include the following.

1) "Complement" receptors on monocytes from patients with RA and SLE should be studied with anti-CR1 and anti-CR3 antibodies to determine whether functional defects are due to diminished numbers of CR1 or CR3 receptors.

2) More sensitive methods should be applied to study whether or not blood monocytes with phagocytic defects contain immune complexes. Radiolabelled antibody to complement components, complement degradation products and immunoglobulin would be a more sensitive probe than immunofluorescence for studying this question.

3) This thesis has not examined whether defective mononuclear phagocytes are found in the tissues and this is clearly relevant both to possible alterations in monocyte traffic as well as to the "clearance" function of macrophages in the joints, liver and spleen. Synovial fluid and skin exudate cells would be accessible for studies of macrophage receptor number and function. Immunochemical studies of monocytes migrating into skin exudates would also help to address the question of whether marginated monocytes contain immune complexes or not.

4) In vitro studies of the modulation of "complement" receptors by immune complexes would also be valuable and in

particular should address whether defects of phagocytosis are more easily induced in monocytes from RA or SLE patients than monocytes from normal controls.

REFERENCES

Abramson N, Gelfand E W, Jandl J H, Rosen F S. (1970)
The interaction between monocytes and red cells. Specificity for IgG subclasses and IgG fragments.
J Exp Med; 132; 1207-1215.

Abrahamson D R, Fearon D T. (1983)
Endocytosis of the C3b receptor of complement within coated vesicles in human polymorphonuclear leucocytes and monocytes.
Lab Invest; 48; 162-168.

Achord D T, Brot F, Gonzalez-Noriega A, Sly W, Stahl P. (1977)
Human b-glucuronidase II Fate of infused human placental b-glucuronidase in the rat.
Paed Res; 11; 816-822.

Achord D T, Brot F, Sly W S. (1977)
Inhibition of the rat clearance system for agalacto-orosomucoid by yeast mannans and by mannose.
Biochem Biophys Res Comm; 77; 409-415.

Ackerman S K, Douglas S D. (1978)
Purification of human monocytes on microexudate coated surfaces.
J Immunol; 120; 1372-1374.

Adams R (1873)
A treatise on rheumatic gout, or chronic rheumatic arthritis of all the joints.
Second edition, John Churchill & Sons, Dublin.
436-444

Agnello V (1978)
Complement deficiency states.
Medicine; 57; 1-23

Agnello V, Mitamura T (1982)
Evidence for detection of low molecular weight DNA: anti-DNA complexes in systemic lupus erythematosus.
Arth & Rheum; 25; 788-792.

Alexander W R M, Bremner J M, Duthie J J R (1960)
Incidence of the antinuclear factor in human sera
Ann Rheum Dis; 19; 338

Alexander M D. (1980)
Specificity of Fc receptors on human monocytes for IgG1 and IgG3.
Int Arch Allerg Appl Immunol; 62; 99-103.

Alexander M D, Leslie R G Q, Cohen S. (1976)
Cytophilic activity of enzymatically derived fragments of guinea pig IgG2.
Eur J Immunol; 6 ;101.

Alexander M D, Andrews J A, Leslie R G Q and Wood N G (1978a).
The binding of human and guinea pig IgG subclasses to homologous macrophage and monocyte Fc receptors.
Immunol; 35; 115-123.

Alper C A, Colten H R, Gear J S S, Rabson A R, Rosen F S. (1976)
Homozygous human C3 deficiency. The role of C3 in antibody prduction, Cls-induced vasopermeability, and cobra venom induced passive haemolysis.
J Clin Invest; 57; 222-229.

Alper C A, Abramson N, Johnston R B (Jr), Jandl J H, Rosen F S. (1970)
Increased susceptibility to infection associated with abnormalities of complement mediated functions and of the third component of complement (C3).
N Eng J Med; 282; 349.

Alspaugh M A, Tan E M. (1976)
Serum antibody in RA reactive with a cell associated antigen: demonstration by precipitation and immunofluorescence.
Arth & Rheum; 19; 711-719.

Alspaugh M A, Henle W. (1979)
Significance of elevated EB virus antibodies in serum or synovial fluid from RA patients.
Arth & Rheum; 22; 587.

Alspaugh M A et al (1979)
Lymphocytes transformed by EB virus: induction of nuclear antigen reactive with antibody in RA.
J Exp Med 147; 1018-1027.

Alspaugh et al (1981)
Elevated levels of antibodies to EB virus antigens in sera and synovial fluids of patients with Rheumatoid Arthritis.
J Clin Invest; 67; 1134-1140.

Amsden A, Evans V, Yoshida T, Cohen S. (1978).
Studies on cellular receptors for lymphokines. I Interaction of chemotactic factors with monosaccharides.
J Immunol; 120; 542-549.

Anderson C L. (1982)
Isolation of the receptor for IgG from a human monocyte cell line (U937) and from human peripheral blood monocytes.
J Exp Med; 156; 1794-1806.

Anderson C L. (1980).
The murine macrophage Fc receptor for IgG2b is lipid dependent.
J. Immunol 125; 538-540.

Anderson C L and Grey H M. (1977).
Solubilisation and partial characterisation of cell membrane Fc receptors.
J. Immunol; 118; 819-825.

Anderson C L and Grey H L. (1978).
Physicochemical separation of two distinct Fc receptors on murine macrophage like cell lines.
J. Immunol; 121; 648-652.

Anwar A R E and Kay A B. (1977a)
Membrane receptors for IgG and complement (C4, C3b, and C3d) on human eosinophils and neutrophils and their relation to eosinophilia.
J Immunol; 119; 976-982.

Anwar A R E and Kay A B. (1977b)
The ECF-A tetrapeptides and histamine selectively enhance humn eosinophil complement receptors.
Nature; 269; 522-524.

Anwar A R E and Kay A B. (1978)
Enhancement of human eosinophil complement receptors by pharmacologic mediators.
J Immunol; 121; 1245-1250.

Arend N P, Mannik M. (1972)
In vitro adherence of soluble immune complexes to macrophages.
J Exp Med; 136; 514.

Arend N P, Mannik M. (1973)
The macrophage receptor for IgG: number and affinity of binding sites.
J Immunol; 110; 1455

Arend W P, Maisoni R J. (1981)
The effect of complement in adherent immune complexes on Fc and C3 receptor expression in human monocytes.
Immunology; 44; 717-725.

Arnaout M A, Melamed J, Tack B F, Colten H R. (1981)
Characterisation of the human complement (C3b) receptor with a fluid phase dimer.
J Immunol; 127; 1348-1354.

Aschoff L. (1924)
Das reticuloendotheliale system.
Ergeb Inn Med und Kinderheilk; 26; 1-118.

Ashwell G and Morell A G. (1974)
The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoprotein.
Adv Enzm Relat Areas Mol Biol; 41; 99-128.

Askenase P W, Hayden J A. (1974)
Cytophilic antibodies in mice contact-sensitised with oxazolone.
Immunology; 27; 563-576.

Atkinson J P, Michael J M, Chaplin H, Parker C W. (1977b)
Modulation of macrophage C3b receptor function by cytochalasin sessitive structures.
J Immunol; 118; 1292-1299.

Atkinson J A, Schreiber A D, Frank M M. (1973)
Effects of corticosteroids and splenectomy on the immune clearance and destruction of erythrocytes.
J Clin Invest; 52; 1509-1517.

Atkinson J P, Frank M M. (1974)
The effect of Bacillus-Calmette-Guerin induced macrophage activation on the in vivo clearance of sensitised erythrocytes.
J Clin Invest; 53; 1742-1749.

Atkinson J P, Frank M M. (1974)
Studies on the in vivo effects of antibody: interaction of IgM antibody and complement in the immune clearance and destruction of erythrocytes in man.
J Clin Invest; 54; 339.

Bagg J, Poxton I R, Weir D M (1981)
The isolation of a lectin-like molecule from Corynebacterium parvum (NCTC 10390)
J Clin Lab Immunol; 6; 165-168.

Banda M J, Clark E J, Werb Z. (1981)
The role of macrophage elastase in the proteolysis of plasma and interstitial proteins.
In: Heterogeneity of mononuclear phagocytes; 286-289.
Eds: Landy M, Foster O.
Publ: Martinuus Nijhoff, The Hague.

Banda M J, Werb Z. (1980)
The role of macrophage elastase in the proteolysis of fibrinoen, plasminogen and fibronectin.
Fed Proc; 39; 1756.

Banda M J, Werb Z. (1980).
Macrophage elastase degradation of immunoglobulins regulates binding to macrophage Fc receptors.
Fed Proc; 39;799.

Banda M J, Werb Z. (1979).
Purification and characterisation of macrophage elastase.
Fed Proc; 38; 1339.

Bardwick P A, Bluestein H G, Zvaifler N J, Depper J M, Seegmiller J E. (1980)
Altered regulation of EB virus induced lymphoblast proliferation in RA lymphoid cells.
Arth & Rheum; 23; 626-632.

Barlin E, Lesen H G, Deimann W, Till G, Resch K, Gemsa D. (1981)
In vivo activation of macrophages by C. parvum, glucna and glycan coplymer: different effects on prostaglandin E release, tumour cytostasis and lymphocyte mitogenicity.
In: Heterogeneity of mononuclear phagocytes; 243-252.
Eds: Foster M, Landy O.
Publ: Martinus Nijhoff; The Hague.

Barcilli U, Rademacher R, Ooi Y M, Ooi B S. (1981).
Modification of glomerular immune complex deposition in mice by activation of the reticulo-endothelial system.
J. Clin. Invest; 67; 20-27.

Barratt M E J, Fell H B, Coombs R R A and Glauvert A M (1977).
The pig synovium (2) some properties of isolated intimal cells.
J. Anat; 123; 47.

Batchelor J R, Welsh K I, Tinoso R M et al (1980)
Hydralllazine induced systemic lupus erythematosus: influence of HLA-DR and sex on susceptibility.
Lancet; 1; 1107-1109.

Baynes J and Wold F. (1976)
Effect of glycosylation on the in vivo circulating half life of ribonuclease.
J Biol Chem; 251; 6016-6024.

Benacerraf B (1978).
Hypothesis to relate the specificity of T-lymphocytes and the activity of I region-specific Ir genes in macrophages and B-lymphocytes.
J. Immunol; 120; 1809.

Benedek T G, Rodnan G P (1982)
A short history of the rheumatic diseases
Bull Rheum Dis; 32; 59-68

Bergholtz B O and Thorsby E (1977)
Macrophage dependent response of immune human T lymphocytes to PPD in vitro.
Scan J Immunol; 6; 779-786.

Berken A, Benacerraf B. (1966)
Properties of antibody cytophilic for macrophages.
J Exp Med; 123; 119.

Berlin R D, Oliver J M. (1978).
Analogous ultrastructure and surface properties during capping and phagocytosis in leucocytes.
J. Cell Biol; 77; 789.

Besana C, Lazzarin A, Carioni F, Carredda F, Moroni M. (1975)
Phagocyte function in systemic lupus erythematosus.
Lancet; 8; 918.

Bianco C, Griffin F M (Jr), Silverstein S C. (1975)
Studies on the macrophage complement receptor. Alteration of receptor function upon macrophage activation.
J Exp Med; 141; 1278-1290.

Biesecker G, Podack E R, Halverson C A, Muller-Eberhard H J (1979).
C5b-9 dimer: Isolation from complement lysed cells and ultrastructural identification with complement dependent membrne lesions.
J. Exp. Med; 149; 448-458

Blaineau C, Gisslebrecht S, Piatier D, Hurot M, Levy J P. (1978)
Swan mice: Autoimmuné mice without xenotropic type C virus production.
Clin Exp Immunol; 31; 276-280.

Blusse van Oud Alblas A and van Furth R (1979).
Origins, kinetics and characteristics of pumonary macrophages in the normal steady state.
J. Exp. Med; 149; 1504.

Bokisch V A, Dierich M P and Muller-Eberhard H J (1975)
Third component of complement (C3) : Structural properties in relation to functions.
Proc Natl Acad Sci USA; 72; 1989-1993.

Bonney R J, Wightman P D, Dahlgren M E, Davies P, Kuehl A, Humes J L (1980b).
Effect of RNA and protein synthesis inhibitors on the releaseof inflammatory mediators by macrophages responding to phorbol myristate acetate.
Biochim Biophys Acta; 633; 410-421.

Bonney R J, Naruns P, Davies P, Humes J L (1979)
Antigen-antibody complexes stimulate the synthesis and release of prostaglandins by mouse peritoneal macrophages.
Prostaglandins; 18; 605-616.

Bonney R J, Davies P L, Kuehl F A, Humes J L. (1981)
Arachidonic acid oxygenation by various populations of macrophages.
In: Heterogeneity of mononuclear phagocytes; 257-262.
Ed: Foster O, Landy M.
Publ: Martinuus Nijhoff, The Hague.

Bonney R J, Davies P, Kuehl Jr. F A and Humes J L (1980)
Arachidonic acid oxygenation products produced by mouse peritoneal macrophages responding to inflammatory stimuli.
J Retic Soc; 28; Suppl; 113s-115s.

Bordet J (1896)
Sur le mode d'action des serums preventifs.
Ann Inst Pasteur Paris 10; 193-219.

Boyle M D P and Borson T (1980).
The terminal stages of immune haemolysis - a brief review.
Mol Immunol; 17; 425-432.

Boyum A. (1968)
Isolation of mononuclear cells and granulocytes from human blood.
Scand J Clin Lab Invest; 21; Suppl 97; 77-89.

Brentjens J R, Andres G A (1982)
The pathogenesis of extrarenal lesions in systemic lupus erythematosus.
Arth & Rheum; 25; 880-886.

Briscoe J C. (1908)
An experimental investigation of the phagocytic action of the alveolar cells of the lung.
J Pathol Bacteriol; 12; 66

Brodie B C (1818)
Pathological and surgical observations on the diseases of the joints.
Longman, London.
18-30

Brown J C and Koshland M E (1977).
Evidence for a long-range conformational change induced by antigen binding to IgM antibody.
Proc. Natl. Acad. Sci. U.S.A.; 74; 5682-5686.

Cairns S A, London A, Mallick N P. (1980)
The value of three immune complex assays in the management of SLE: an assessment of immune complex levels, size and immunochemical properties in relation to disease activity and manifestations.
Clin Exp Immunol; 40; 273-282.

Capo C, Bongrand P, Benoliel A-M and Depieds R (1979).
Non-specific recognition in phagocytosis: ingestion of aldehyde-treated erythrocytes by rat peritoneal macrophages.
Immunol; 36; 501-508.

Capron A, Dessaint J-P and Capron M (1975).
Specific IgE antibodies in immune adherence of normal macrophages to Schistosoma mansoni schistosomules.
Nature; 253; 474-475.

Cardella C J, Davies P and Allison A C (1974).
Immune complexes induce slective release of lysosomal enzymes by macrophages.
Nature (London); 247; 46-48.

Carlo J R, Ruddy S, Studer E J and Conrad D H (1979).
Complement receptor binding of C3b-coated cells treated with C3b onactivator, BlH globulin and trypsin.
J. Immunol; 123; 523-528.

Carson D A, Lawrence S, Slaughter L, Vaughan J H (1979)
Immunochemical properties of anti-IgG antibodies.
In: Immunopathogenesis of rheumatoid arthritis.
Ed: Panayi G S; Reed Books, Chertsey, UK.
51-55.

Catalano M A, Carson D, Slovin S , Richman D, Vaughan J (1979)
Antibody to EB virus determined antigens in normal subjects and in patients with seropositive Rheumatoid Arthritis.
Proc Nat Acad Sci (USA); 76; 5825-5828.

Charcot J-M (1881)
Clinical lectures on the diseases of old age.
Translated by Hunt L H.
W Wood, New York.
101-102.

Claus D R, Siegel J, Petras K (1977).
Interactions of C-reactive protein with the first component of human complement.
J Immunol; 119; 187-192.

Cohen A S, Canoso J J (1972)
Criteria for the classification of SLE-status 1972.
Arth & Rheum; 15; 540.

Cohn Z A, Benson B. (1965)
The differentiation of mononuclear phagocytes.
J Exp Med; 121; 153-164.

Cohn Z A, Weiner E. (1963)
The particulate hydrolases of macrophages.
J Exp Med; 118; 991-1008.

Cohn Z A (1970).
Endocytosis and intracellular digestion.
In Mononuclear Phagocytes. pp 121-132. (R. van Furth, ed.,
Blackwell Scientific Publications. Oxford, Edinburgh).

Cohn Z (1978).
The activation of monunuclear phagocytes: Fact, fancy and future.
J. Immunol; 121; 813-816.

Cole B C, Cassell G H (1980)
Mycoplasma infections as models of chronic joint inflammation.
Arth & Rheum; 22; 1375-1381.

Colomb M, Porter R R. (1975).
Characterisation of a plasmin-digest fragment of rabbit
immunoglobulin gamma that binds antigen and complement.
Biochem. J. 145; 117-183.

Conn D L, Mc Duffie F C, Dyck P J. (1972)
Immunopathologic study of sural nerves in rheumatoid arthritis.
Arth & Rheum; 15; 135-143.

Cook W J, Bugg E C. (1975)
Calcium-carbohydrate bridges composed of uncharged sugars.
Structure of a hydrated calcium bromide complex of alpha-fucose.
Biochim et Biophys Acta; 389; 428-435.

Coupland K, Leslie R G Q. (1983)
The expression of Fc receptors on guinea pig peritoneal macrophages and neutrophils.
Immunology; 48; 647-656.

Cox D W, Huber O (1980)
Association of severe RA with heterozygosity for alphal-antitrypsin deficiency
Clin Gen; 17; 153-160

Czop J K, Fearon D T and Austen K F (1978a).
Membrane sialic acid on target particles modulates their phagocytosis by a trypsin-sensitive mechanism on human monocytes.
Proc. Natl. Acad. Sci. U.S.A.; 75; 3831-3835.

Czop J K, Fearon D T, Austen K F (1978b).
Opsonin independent phagocytosis of activators of the alternative complement pathway by human monocytes.
J Immunol; 120; 1132-1138.

Daems, W Th and van der Rhee H J (1980).
Peroxidase and catalse in monocytes, macrophages, epitheliod cells and giant cells of the rat.
In Mononuclear Phagocytes. Functional Aspects. Part I.pp 43-60. (R. van Furth, ed; Martinus Nijhoff Publishers by The Hague, The Netherlands).

Dannenberg A M (1975).
Macrophages in inflammation and infection.
N. Engl. J. Med.; 293; 489.

Dawson M H, Olmstead M, Boots R H. (1932)
Agglutination reactions in rheumatoid arthritis. I. Agglutination reactions with streptococcus haemolyticus.
J Immunol; 23; 187-204.

Day J F, Thornburg R W, Thorpe S R, Baynes J W. (1980)
Carbohydrate mediated clearance of antibody-antigen complexes from the circulation. The role of high mannose oligosaccharides in the hepatic uptake of IgM -antigen complexes.
J Biol Chem; 255; 2360-2365.

Dayer J-M, Krane S M, Russell R G G, Robinson D R.(1976)
Production of collagenase and prostaglandins by isolated adherent rheumatoid synovial cells.
Proc. Natl. Acad. Sci. USA 1976; 73; 945-9.

Dayer J-M, Passwell J H, Schneeberger E E, Krane S M.(1980)
Interactions among rheumatoid synovial cells and monocyte-macrophages: Production of collagenase-stimulating factor by human monocytes exposed to concanavalin A or immunoglobulin Fc fragments.
J. Immunol. 1980; 124; 1712-20.

Dayer J-M, Robinson D R, Krane S M.(1977)
Prostaglandin production by rheumatoid synovial cells. Stimulation by a factor from human mononuclear cells.
J. Exp. Med. 1977; 145; 1399-404.

Dayer J-M, Goldring S R, Robinson D R, Krane S M. (1979).
Effects of human mononuclear cell factor on cultured rheumatoid synovial cells. Interactions of prostaglandin E2 and cylcic adenosine 3', 5'-monophosphate.
Biochim Biophys. Acta. 1979; 586; 87-105.

Dayer J-M, Breard J, Chess L, Krane S M. (1979a).
Participation of monocyte-macrophages and lymphocytes in the production of a factor that stimulates collagenase and prostaglandin release by rheumatoid synovial cells.
J. Clin. Invest. 1979; 64; 1386-92.

Dayer J-M, Stephenson M L, Schmidt E, Karge W, Krane S M.(1981)
Purification of a factor from human monocyte-macrophages which stimulates the production of collagenase and prostaglandin E2 by cells cultured from rheumatoid synovial tissues.
FEBS Lett 1981; 124; 253-6.

De Ceular C, Papazoglou S, Whaley K. (1980)
Increased biosynthesis of complement components by cultured monocytes, synovial fluid macrophages and synovial membrane cells from patients with RA.
Immunol; 41; 37-43.

DeChatelet L R, McCall C E, Shirley P S. (1973).
Creatine phosphokinase activity in rabbit alveolar macrophages.
Inf & Immun; 7; 29.

Deporter D A (1979).
The role of the macrophage in collagen resorption during chronic inflammation. A new look at an old hypothesis.
Agents and Action; 9; 168.

Depper J M, Zvaifler N J (1981)
Epstein-Barr virus: Its relationship to the pathogenesis of Rheumatoid Arthritis.
Arth & Rheum; 24; 755-761.

Depper J M, Zvaifler N J, Bluestein H G (1982)
Oropharyngeal EB virus excretion in Rheumatoid Arthritis.
Arth & Rheum; 25; 427-431.

Dessaint J P, Torpier G, Capron M, Bazin H, Capron A. (1979)
Cytophilic binding of IgE to the macrophage.
Cell Immunol; 46; 12-23.

Diamond B and Yelton D E (1981).
A new Fc receptor on mouse macrophages. Binding of IgG3.
J. Exp. Med; 150; 721-726.

Diamond B, Birshtein B K and Scharff M D (1979).
Site of binding of mouse IgG2b to the Fc receptor on mouse macrophages.
J. Exp. Med; 150; 721-726.

Diamond B, Bloom B R and Scharff M D (1978).
The Fc receptors of primary and cultured phagocytic cells studied with homogeneous antibodies.
J. Immunol; 121; 1329-1333.

Dias da Silva W, Eisele J W and Lepow I H. (1967).
Complement as a mediator of inflammation. III. Purification of the activity with anaphylotoxin properties generated by interaction of the first four components of complement and its identification as a cleavage product of C'3.
J. Exp. Med. 126; 1027-1048.

Dierich M P. (1976)
C3 receptors on lymphoid cells: solubilisation by KBr and partial purification by immunoadsorption.
Fed Proc; 35; 1700 (abstr).

Dierich M P, Ehlen T L, Mussel H H. (1981)
Description of a simple specific and sensitive test for the detection of detergent solubilised C3b receptor.
Immunobiology; 160; 241-247.

Dierich M P, Scheiner O, Mussel H H, Hamman K P, Schopf R E, Schultz T. (1982)
Characterisation of complement receptors.
Molec Immunol; 19; 1255-1265.

Dierich M P, Schmitt M. (1980)
Raji cells have blH receptor activity which enhances C3b binding.
J Immunol; 124; 1520.

Dinarello C A, Marnoy S O, Rosenwasser L J. (1983)
Role of arachidonic metabolism in the immunoregulatory function of human Leucocyte pyrogen/lymphocyte activating factor/ interleukin 1.
J Immunol; 130; 890-895.

Dingle J T, Horsfield, P, Fell H B and Barratt M E J (1975).
Breakdown of proteoglycan and collagen induced in pig articular cartilage in organ culture.
Ann. Rheum. Dis; 34; 303.

Dingle J T (1981).
The role of catabolin in the control of the degradation of articular cartilage.
New research developments in osteoarthrosis; ed. J G Peyron; 31-33.

Dingle J T and Dingle T T.
The site of cartilage matrix degradation.
Biochem J. 190; 431-438; 1980.

Dobson N J, Lambris J D, Bleau S A, Ross G D. (1981).
Roles of human neutrophil complement receptors and blH in the release of superoxide anion.
Proc Nat Acad Sci USA; 40; 1014.

Dobson N J, Lambris J D, Ross G D. (1981)
Characteristics of isolated erythrocyte complement receptor tpe one (CRl, C4b-C3b receptor) and CRl specific antibodies.
J Immunol; 126; 693-698.

Dorrington K J, Klein M H. (1982)
Binding sites for Fc receptors on immunoglobulin G and factors influencing their expression.
Molec Immunol; 19; 1215-1221.

Doughaday C C and Douglas S D. (1976)
Membrane receptors on rabbit and human pulmonary alveolar macrophages.
J Retic Soc; 19; 37-45.

Douglas S. (1976)
Human monocyte spreading in vitro - inducers and effects on Fc and C3 receptors.
Cell Immunol; 21; 344-349.

Dykman T R, Cole J L, Iida K, Atkinson J P (1983)
Polymorphism of human erythrocyte C3b/C4b receptor.
Proc Nat Acad Sci; 80; 1698-1702.

Eaves A C and Bruce W K (1974).
In vitro production of colony-stimulating activity. I Exposure of mouse peritoneal cells to endotoxin.
Cell Tissue Kinetics; 7; 19-30.

Egan R W, Paxton J, Kuehl F A (1976).
Mechanism for self deactivation of prostaglandin synthetase.
J Biol Chem; 251; 7329-7355.

Ehlenberger A G, Nussenzweig V. (1977)
The role of membrane receptors for C3b and C3d in phagocytosis.
J Exp Med; 145; 357.

Ehrlich P (1900).
On immunity with special reference to cell life (Croonian Lecture).
Proc Roy Soc London; 66; 424-448.

Elkon K B, Sewell J F, Ryan P F J, Hughes G R V (1980).
Splenic function in nonrenal systemic lupus erythematosus.
Am J Med; 69; 80-82.

Elkon et al (1982)
Evaluation of heat damaged and IgG coated red cells for testing reticulo endothelial function.
J Immunol Meth; 55; 253-263.

Ellner J J. (1981)
Suppressor cells of man.
Clin Immunol Rev; 1; 119.

Elson C J, Crawford D M, Bucknall R C, Allen C, Thomson J L, Hall N D, Bacon P A, Epstein M A (1979)
Infection with EB virus and Rheumatoid Arthritis
Lancet; 1; 105.

Emlen W, Mannik M, (1978).
Kinetics and mechanisms for removal of circulating single stranded DNA in mice.
J Exp Med; 147; 684-699.

Evanson J M, Jeffrey J J and Krane S M (1967).
Human collagenase : identification and characterization of an enzyme from rheumatoid synovium in culture.
Science; 158; 499.

Fauci A S (1982)
Human B-lymphocyte function: cell triggering and immunoregulation
J Inf Dis; 145; 602-612.

Fearon D T, Austen F K, Ruddy S (1973).
Formation of a haemolytically active cellular intermediate by the interaction between properdin factors B and D and the activated third component of complement.
J. Exp. Med; 138; 1305-1313.

Fearon D T and Austen K F (1976).
Properdin: Binding to C3b and stabilisation of the C3b-dependent C3 convertase.
J. Exp. Med; 142; 856-863.

Fearon D T and Austen K F (1980).
The alternative pathway of complement - a system for host resistance to microbial infection.
NEJ Med; 303; 259-263.

Fearon D T (1978).
Regulation by sialic acid of blH-dependent decay-dissociation of amplification C3 convertase of the alternative complement pathway.
Proc. Nat. Acad. Sci; 75; 1971-1975.

Fearon D T and Austen K F.(1977)
Activation of the alternative complement pathway due to resistance of zymosan bound amplification convertase to endogenous regulatory mechanisms.
Proc Natl Acad Sci; 74; 1683-1687.

Fearon D T (1980)
Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte and monocyte.
J exp Med; 152; 20-30.

Fearon D T (1979)
Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from the human erythrocyte membrane.
Proc Natl Acad Sci USA; 76; 5867-5871

Fearon D T, Kaneko I, Thomson G G. (1981)
Membrane distribution and adsorptive endocytosis by C3b receptors on human polymorphonuclear leucocytes.
J Exp Med; 153; 1615-1628.

Fearon D T, Collins L A. (1983)
Increased expression of C3b receptors on polymorphonuclear leucocytes induced by chemotactic factors and purification procedures.
J Immunol; 130; 370-375.

Fell H B (1978).
Synoviocytes.
J. Clin. Pathol; 31 (Suppl. Royal College of Pathology); 12; 14.

Fell H B and Jubb R J (1977).
The effect of synovial tissue on the breakdown of articular cartilage in organ culture.
Arth. Rheum; 20; 1359.

Fell H B and Barratt M E J (1973).
The role of soft connective tissue in the breakdown of pig articular cartilage cultivated in the presence of complement-sufficient antiserum to pig erythrocytes (1). Histological changes.
Int. Arch. Allery Appl. Immunol; 44; 441.

Fell H B, Glauvert, A M, Barratt M E J and Green R (1976).
The pig synovium (1) the intact synovium in vivo and in organ culture.
J. Anat; 122; 663.

Felty A R (1924)
Chronic arthritis in the adult, associated with splenomegaly and leucopenia: a report of 5 cases of an unusual clinical syndrome.
J Hopkins Hosp Bull; 35; 16-20.

Ferrel P B, Aitcheson C T, Pearson G R, Tan E M (1981)
Seroepidemiological study of relationships between EB virus and Rheumatoid Arthritis.
J Clin Invest; 67; 681-687.

Finbloom D S, Plotz P H (1979a).
Studies of reticulo endothelial function in the mouse with model immune complexes. I. Serum clearance and tissue uptake in normal C3H mice.
J. Immunol; 123; 1594-1599.

Finbloom D S, Plotz P H (1979b).
Studies of reticulo endothelial function in the mouse with model immune complexes. II. Serum clearance tissue uptake and reticulo endothelial saturation in NZB/W mice.
J. Immunol; 123; 1600-1603.

Fischer A, Durandy A and Griscelli C (1981).
Role of prostaglandin E2 in the induction of nonspecific T lymphocyte suppressor activity.
J. Immunol; 126; 1452-1455.

Fischer A, Ballet J, Criscelli C. (1978)
Specific inhibition of in vitro candida-induced lymphocyte proliferation by polysaccharidic antigens present in the serum of patients with chronic mucocutaneous candidiasis.
J Clin Invest; 62; 1005-1013.

Fogel M, Altevogt P, Schirrmacher V. (1983)
Metastatic potential severely altered by changes in tumour cell adhesiveness and cell-surface sialylation.
J Exp Med; 157; 371-376.

Ford M J, Hurst N P, Nuki G (1983)
Reactive arthritis-infectious agents and genetic susceptibility in the pathogenesis of seronegative arthritis.
Scot Med J; 28; 34-41.

Ford P M (1975).
Glomerular localisation of aggregated protein in mice: effect of strain differences in relationship to systemic macrophage function.
B J Exp Path; 56; 307-313.

Ford P M and Kosatka I (1979)
In situ formation of antigen-antibody complexes in the mouse glomerulus.
Immunology; 38; 473-479.

Frank M M, Hamburger M I, Lawley I J, Kimberley R P, Plotz P H. (1979)
Defective reticuloendothelial system Fc receptor function in systemic lupus erythematosus.
N Eng J Med; 300; 518-23.

Freimer N B, Ogmundsdottir H M, Blackwell C C, Sutherland I W, Graham L and Weir D M. (1978).
The role of cell wall carbohydrates in binding of micro-organisms to mouse peritoneal exudate macrophages.
Acta Path Micrbiol Scand Sect B; 86; 53-57.

Friedman R M, Preble O, Black R, Harrell S (1982)
Interferon production in patients with SLE.
Arth & Rheum; 25; 822-803.

Fries L F, Hall R P, Lawley T J, Crabtree G R, Frank M. (1982)
Monocyte receptors for the Fc portion of IgG studied with monomeric human IgG1: normal in vitro expression of Fc receptors in HLA-B8/Drw3 subjects with defective Fc mediated in vivo clearance.
J Immunol; 129; 1293-1049.

Fujita T, Gigli I, Nussenzweig V (1978).
Human C4 binding protein. II. Role in proteolysis of C4b by C3b-inactivator.
J. Exp. Med; 148; 1044-1051.

van Furth R, Raeburn J A, van Zwet T L. (1979).
Characteristics of human mononuclear phagocytes.
Blood; 54; 485-500.

van Furth R. (1981)
Development of mononuclear phagocytes.
In: Forster O, Landy M. eds. Heterogeneity of mononuclear phagocytes. Amsterdam, Academic Press; 1981; 3-10.

van Furth R. (1980).
Cells of the mononuclear phagocyte system. Nomenclature in terms of sites and conditions.
In: van Furth R, ed. Mononuclear Phagocytes: Functional aspects. Part I. Martinus Nijhoff, The Hague; 1-30.

van Furth R, Diesselhoff-den Dulk M C, Raeburn J A, van Zwet T L, Crofton R, Blusse van Oud Alblas A.(1980)
Characteristics, origin and kinetics of human and murine mononuclear phagocytes.
In: van Furth R, ed. Mononuclear Phagocytes: Functional aspects Part 1. The Hague, Martinus Nijhoff; 279-98.

van Furth R (1976).
An approach to the characterization of mononuclear phagocytes involved in pathological processes.
Agents and Actions; 6; 91.

van Furth R (1970).
The effect of glucocorticsteroids on the kinetics of monocytes and peritoneal macrophages.
In: Mononuclear phagocytes; pp255-264.
Ed: van Furth R.
Blackwell Scientific Publications, Oxford.

Garrod A B. (1892)
The great practical importance of separating rheumatoid arthritis from gout.
Lancet; 2; 1033-1037.

Gemsa D, Seitz M, Kramer W, Grimm W, Till G, Resch K. (1979)
Ionophore A21387 raises cyclic AMP levels in macrophages by stimulating prostaglandin E formation.
Exp Cell Res; 118; 55-62

Gergely J, Erdei A, Sandor M, Sarmay G, Uher F. (1982)
The Fc receptor model of membrane cytoplasmic signalling.
Mol Immunol; 19; 1223-1228.

Ghebrehiwet B, Muller-Eberhard H J. (1979)
C3e: an acidic fragment of human C3 with leucocytosis inducing activity.
J Immunol; 123; 616-621.

Gigli I, Nelson R A (Jr) (1968)
Complement dependent immune phagocytosis. I. Requirements for C'1, C'4, C'2 and C'3.
Exp Cell Res; 51; 45-67

Glass E J, Kay A B. (1980)
Enhanced expression of human monocyte (C3b) receptors by chemoattractants.
Clin Exp Immunol; 39; 768-775.

Glass E J, Stewart J and Weir D M (1981b).
Presence of bacterial binding "lectin-like" receptors on phagocytes.
Immunology; 44; 529-534

Glynn L E, Reading C A.(1981)
Immunological investigation of connective tissue diseases.
Edinburgh, London. Churchill Livingstone; 62-63.

Goetzl E J, Derian C and Valone F H (1980).
The extracellular and intracellular roles of hydroxy-eicosatetraenoic acids in the modulation of polymorphonuclear and macrphage function.
J. Retic. Soc; 28; Suppl. Dec; 105s-111s.

Goldring S R, Dayer J-M, Krane S M. (1980)
Hormone responses modulated by cell-cell interactions.
Calcif Tissue Int; 72.

Goldstein J L, Dann S E, Brunschede G Y, Brown M S. (1975)
Genetic heterogeneity in familial hypercholesterolaemia; evidence for two different mutations affecting functions of LDL receptor.
Proc Nat Acad Sci; 72; 1092.

Goldstein I M, Kaplan H B, Radin A and Frosch M (1976).
Independent effects of IgG and complement upon human polymorphonuclear leukocyte function.
J. Immunol; 117; 1282-1287.

Goldstein I M, Roos D, Kaplan H B and Weissmann G (1975).
Complement and immunoglobulin stimulate superoxide production by human leucocytes independently of phagocytosis.
J. Clin. Invest. 56; 1155.

Goodwin J S, Bankhurst A D and Messner R P (1977).
Suppression of human T-cell mitogenesis by prostaglandin.
Existence of a prostaglandin-producing suppressor cell.
J. Exp. Med; 146; 1719-1734.

Goodwin J S, Messner R P Bankhurst A D, Peak G T, Saiki J H, Williams R C. (1977)
Prostaglandin producing suppressor cells in Hodgkins.
N Eng J Med; 297; 963-968.

Gordon D, Bray M A and Morley J (1976).
Control of lymphokine secretion by prostaglandins.
Nature; 262; 401-402.

Gordon S, Unkeless J C and Cohn Z A (1974).
Induction of macrophage plasminogen activator by endotoxin stimulation and phagocytosis: evidence for a two-stage process.
J. Exp. Med; 140; 995-1010.

Gordon S, Newman W and Bloom B (1978).
Macrophage proteases and rheumatic diseases; regulation of plasminogen activator by thymus-derived lymphocytes.
Agents and Actions; 8;1-2.

Gordon D, Bray M A and Morley J (1976)
Control of lymphokine secretion by prostaglandins.
Nature (London); 262; 402.

Gordon P A, Davis P, Russell A S, Coates, J E, Rothwell R S, Leclerq S M. (1981)
Splenic reticuloendothelial function in patients with active rheumatoid arthritis.
J Rheum; 8; 490-493.

Gotze O, Sundsmo J S. (1981)
A role for membrane-associated complement proteins in the spreading of human mononuclear phagocytes.
In: Forster O, Landry M, eds; Heterogeneity of mononuclear phagocytes; pp115-121; Academic Press, London, New York.

Gotze O and Muller-Eberhard H J (1976).
The alternative pathway of complement activation.
Adv. Immunol; 24; 1-35.

Goup T, Schotte C, van Furth R (1975)
Identification and characterisation of the monoblast in mononuclear phagocyte colonies grown in vitro.
J Exp Med; 141; 118P-1199.

Grahame R, Armstrong R et al (1981)
Isolation of Rubella virus from synovial fluid in five cases of seronegative arthritis.
Lancet; 2; 649-651.

Green G M (1970).
In defense of the lung.
Am. Rev. Resp. Dis; 102; 691-703.

Greenwood B M, Whittle H C. (1976)
The pathogenesis of meningococcal arthritis
In: Dumonde D C, ed; Infection and immunology of rheumatic diseases; ppll9-127; Blackwell, Oxford.

Griffin (Jr) F M (1980).
Effects of soluble immune complexes on Fc receptor-and C3b receptor-mediated phagocytosis by macrophages.
J. Exp. Med; 152; 905-919.

Griffin J A, Griffin F M (Jr). (1979)
Augmentation of macrophage complement receptor function in vitro. I. Characterisation of the cellular interactions required for the generation of a T-lymphocyte product that enhances macrophage complement receptor function.
J Exp Med; 150; 653-675

Griffin (Jr) F M and Griffin J A (1980).
Augmentation of macrophage complement receptor function in vitro. II. Characterization of the effects of a unique lymphokine upon the phagocytic capabilities of macrophages.
J. Immunol; 125; 844-849.

Griffin (Jr) F M, Bianco C and Silverstein S C (1975).
Characterization of the macrophage receptor for complement and demonstration of its functional independence from the receptor for the Fc portion of immunoglobulin G.
J. Exp. Med; 141; 1269-1277.

Griffin (Jr) F M, Griffin J A and Silverstein S C (1976).
Studies on the mechanism of phagocytosis. II The interaction of macrophages with anti-immunoglobulin IgG-coated bone marrow-derived lymphocytes.
J. Exp. Med; 144; 788-809.

Griffin (Jr) F M, Griffin J A, Leider J E and Silverstein S C (1975b).
Studies on the mechanism of phagocytosis. I Requirements for circumferential attachment of particle bound ligands to specific receptors on the macrophage plasma membrane.
J. Exp. Med.; 142; 1263-1282.

Griffin (Jr) F M, Silverstein S C (1974)
Segmental response of the macrophage plasma membrane to a phagocytic stimulus.
J Exp Med; 139; 323.

Grimm W, Seitz M, Kirchner H, Gemsa D. (1978)
Prostaglandin synthesis in spleen cell cultures of mice injected with C. parvum.
Cell Immunol; 40; 419-426.

Haakenstad A O, Mannik M. (1974)
Saturation of the reticuloendothelial system with soluble immune complexes.
J Immunol; 112; 1939-1948.

Haakenstad A O, Mannik M. (1976)
The disappearance kinetics of soluble immune complexes prepared with reduced and alkylated antibodies.
Lab Invest; 107; 63-75.

Haakenstad A O, Case J B, Mannik M. (1975)
Effect of cortisone on the disappearance kinetics and localisation of soluble immune complexes.
J Immunol; 114; 1153-1160.

Hahn B H, Bagby M K, Osterland C K. (1973)
Abnormalities of delayed hypersensitivity in systemic lupus erythematosus.
Am J Med; 55; 25-31.

Hamburger M, Haralamdos M, Moutsopoulos M D, Lawley T J, Frank M M. (1979)
Sjogrens syndrome: A defect in reticuloendothelial system Fc receptor specific clearance.
Ann Int Med; 91; 534-538.

Hamburger M I, Field T R, Lawley T J, Hall R, Kimberley R P, Plotz P H, Frank M M. (1981)
Reticuloendothelial system C3b receptor function in systemic lupus erythematosus.
Arth & Rheum; 24; Abstr 141.

Hamburger M I, Lawley R J, Kimberley R P, Plotz P H, Frank M M. (1982)
A serial study of splenic reticuloendothelial system Fc receptor functional activity in systemic lupus erythematosus.
Arth & Rheum; 25; 48-54.

Hamerman D, Janis R, Smith C (1967)
Cartilage matrix depletion by rheumatoid synovial cells in tissue culture.
J Exp Med; 126; 1005-1012.

Hanto D W, Frizzera G, Gajl-Peczalska K J, Sakamoto K, Balfour H H, Simmons R L, Najarian J S. (1982)
Epstein Barr virus induced lymphoma after renal transplantation
N Eng J Med; 306; 913-918.

Hargraves M M, Richmond H, Morton R. (1948)
Presentation of two bone marrow elements: the "tart" cell and the "LE" cell.
Proc Staff Meet Mayo Clin; 23; 25-28

Harris E D Jr, Evanson J M, Dibona D R, Krane S M. (1970)
Collagenase and rheumatoid arthritis.
Arthritis Rheum; 13; 83-94.

Harris E D Jr, DiBona D R, Krane S M. (1969)
Collagenases in human synovial fluid.
J. Clin. Invest; 48; 2104-13.

Harris E D Jr, Krane S M.(1974)
Collagenases.
N. Engl. J. Med; 291; 557-63; 605-9; 652-61.

Harrison R A and Lachman P J (1980).
The physiological breakdown of the third component of human complement.
Mol. Immunol; 17; 9-20.

Hart H, Norval M. (1980)
Search for viruses in rheumatoid macrophage rich synovial cell populations.
Ann Rheum Dis; 39; 159-163.

Hart H, McCormick J N, Marmion B P. (1979)
Viruses and lymphocytes in RA II. Examination of lymphocytes and sera from patients with rheumatoid arthritis for retrovirus infection.
Ann Rheum Dis; 38; 514-525.

Hartwig J H and Stossel T P (1975).
Isolation and properties of actin, myosin and a new actin-binding protein in rabbit alveolar macrophages.
J. Biol. Chem; 250; 5696-5705.

Hartwig J H, Davies W A, Stossel T P. (1977)
Evidence for contractile protein translocation in macrophage spreading, phagocytosis and phagolysosome formation.
J Cell Biol; 75; 936.

Hartwig J H, Yin H L and Stossel T P (1980).
Contractile proteins and the mechanism of phagocytosis in macrophages.
In Mononuclear Phagocytes. Functional Aspects. Part II. pp 971-996. (R. van Furth, ed; Martinus Nijhoff Publishers by The Hague, The Netherlands).

Haserick J R, Lewis L A, Bortz D W. (1950)
Blood factor in acute disseminated lupus erythematosus. I. Determination of gamma globulin as specifc plasma fraction.
Am J Med Sci; 219; 660-663.

Haskill J S and Fett J W (1976).
Possible evidence for antibody-dependent macrophage-mediated cytotoxicity directed against murine adenocarcinoma cells in vivo.
J. Immunol; 117; 1992-1998.

Hay F C, Torrigiani G, Roitt I M. (1972)
The binding of human IgG subclasses to human monocytes.
Eur J Immunol; 2; 257

Henderson J M, Bell D A, Hart M, Chamberlain M J. (1981)
Reticuloendothelial function in rheumatoid arthritis: correlation with disease activity and circulating immune complexes.
J Rheum; 8; 486-489.

Henle W, Henle G. (1981)
Epstein Barr virus specific serology in immunologically compromised individuals
Cancer Res; 41; 4222-4225

Heusser C H, Anderson C L, Grey H M. (1977)
Receptors for IgG:subclass specificity of receptors on different mouse cell types and the definition of two distinct receptors on a mouse macrophage cell line.
J Exp Med; 145; 1316.

Higgins T J, Parish C R, Hogarth P M, McKenzie I F C, Hammerling G J. (1980)
Demonstration of carbohydrate and protein determined Ia antigens by monoclonal antibodies.
Immunogenetics; 11; 467-482.

Hirata F, Axelrod J. (1980)
Phospholipid methylation and biological signal transmission.
Science; 209; 1082-1090.

Hirata F, Corcoran B A, Venkatasubramanian K, Schiffman E, Axelrod J. (1979)
Chemoattractants stimulate degradation of methylated phospholipids and release of arachidonic acid in rabbit leucocytes.
Proc Nat Acad Sci USA; 2640-2643

Hoch S, Schur P H. (1981)
Monocyte receptor function in patients with rheumatoid arthritis
Arth & Rheum; 24; 1268-1277.

Hoffman E M. (1968)
Inhibition of complement by a substance isolated from human erythrocytes.
Immunochem; 6; 391-403 & 405-419.

Hoffstein S T (1979).
Ultrastructural demonstration of calcium loss from local regions of the plasma membrane of surface stimulated human granulocytes.
J Immunol; 123; 1395-1402.

Hoffsten P, Swerdlin A, Bartell M et al (1979)
Reticuloendothelial and mesangial function in murine immune complex glomerulonephritis.
Kidney Int; 15; 144-159.

Holubar K (1980)
Terminology and iconography of lupus erythematosus: a historical vignette.
Am J Dermatopathol; 2; 239-242.

Holt P J A, Davies M G, Saunders K C, Nuki G. (1980)
Pyoderma gangrenosum. Clinical and laboratory findings in 15 patients with special reference to polyarthritis.
Medicine; 59; 114-132.

Horwitz M A (1980).
The roles of the Fc and C3 receptors in the phagocytosis and killing of bacteria by human phagocytes.
J. Retic. Soc; 28; Suppl. Dec; 17-26s.

Horwitz D A. (1972)
Impaired delayed hypersensitivity in sytemic lupus erythematosus.
Arth & Rheum; 15: 353-9.

Horwitz D A. (1982)
Cellular immunity and rheumatic disease.
In: Panayi G, ed; Scientific basis of rheumatology; pp61-71;
Churchill Livingstone

Horwitz D A, Silverstein S C (1980)
Influence of the Escherichia coli capsule on complement fixation and on phagocytosis and killing by human phagocytes.
J Clin Invest; 65; 82-94

Horwitz D A, Steagall R V Jr. (1972)
The development of macrophages from large mononuclear cells in the blood of patients with inflammatory disease.
J Clin Invest; 51; 760-768.

Hovi T, Mosher D and Vaheri A (1977).
Cultured human monocytes synthesise and secrete a2-macroglobulin.
J. Exp. Med; 145; 1580-1589.

Howard J. (1978)
H-2 and HLA antigen structure.
Nature; 27; 607-608.

Howard J B, Benacerraf B. (1961)
Properties of macrophage receptors for cytophilic antibodies.
B J Exp Path; 47; 193

Huber H, Polley M J, Linscott W D, Fudenburg H H and Muller-Eberhard H J. (1968)
Human monocytes: distinct receptor sites for the third component of complement and for immunoglobulin G.
Science; 162; 1281-83.

Huber H, Douglas S D, Nusbacher J, Kochwa S, Rosenfield R E. (1971)
Subclass specificity of IgG for human monocyte receptor sites.
Nature (London); 229; 419

Huber H, Fudenberg H H. (1968)
Receptor sites of human monocytes for IgG.
Int Arch All Appl Immunol; 34; 18.

Humbert J R, Fishman C B, Weston W L, Dearmey R A, Thoren C H. (1976)
Frequency of the carrier state for X-linked chronic granulomatous disease among females with lupus erythematosus.
Clin Genet; 10; 16.

Humes J L, Burger S, Galavage M, Kuehl F A (Jr), Wightman P D, Dahlgren M E, Davies P, Bonney R J. (1980)
The diminished production of arachidonic acid oxygenation products by elicited mouse peritoneal macrophages: possible mechanisms.
J Immunol; 124; 2110-2116.

Hurst N P, Nuki G. (1981)
Evidence for a defect of complement mediated phagocytosis by monocytes from patients with rheumatoid arthritis and cutaneous vasculitis.
B M J; 282; 2081-2083.

Hurst N, Nuki G, Wallington T. (1983)
Functional defects of monocyte C3b receptor mediated phagocytosis in rheumatoid arthritis: Evidence for an association with the appearance of a circulating population of non-specific esterase negative mononuclear phagocytes.
Ann Rheum Dis; In Press.

Iida K, Nussenzweig V. (1981)
Complement receptor is an inhibitor of the complement cascade.
J Exp Med; 153; 1138-1150.

Iida K, Mornaghi R, Nussenzweig V (1982)
Complement receptor (CR1) deficiency in erythrocytes from patents with SLE.
J Exp Med; 155; 1427-1438.

Izui S, McConahey P J, Theofilopoulous A N, Dixon F J. (1978)
Association of retroviral gp70-antigp70 immune complexes with murine SLE.
J Exp Med; 149; 1099-1110.

Jacob H S, Craddock P R, Hammerschmidt D E, Moldow C F (1980)
Complement induced granulocyte aggregation.
N Eng J Med; 302; 789-794.

Jaffe C J, Vierling J M, Jones E, Lawley T J, Frank M M. (1978)
Receptor specific clearance by the reticuloendothelial system in chronic liver diseases: Demonstration of defective C3b-specific clearance in primary biliary cirrhosis.
J Clin Invest; 62; 1069-1077.

James O, Macklon A F, Watson A J (1981)
Primary biliary cirrhosis - a revised clinical spectrum.
Lancet; 1278-1281.

Jancik J M, Shauer R, Andres K H and von During M (1978).
Sequestration of neuraminidase-treated erythrocytes. Studies on topographic, morphologic and immunologic aspects.
Cell Tissue Res; 186; 209-226.

Jarcho S (1957)
Notes on the early modern history of lupus erythematosus.
J Mt Sinai Hosp; 24; 939-944.

Jesaitis A J, Cochrane C G. (1983)
Editorial- Receptor mediated endocytosis, host defense and inflammation.
Lab Invest; 48; 117-119.

Jessop J D, Vernon-Roberts B, Harris J. (1973)
Effects of gold salts and prednisolone on inflammatory cells. I Phagocytic activity of macrophages and polymorphs in inflammatory exudates studied by a "skin window" technique in rheumatoid and control patients.
Ann Rheum Dis; 32; 294-300.

Jimenez R A H, Haakenstad A O, Mannik M. (1983)
Hepatic uptake of small-latticed immune complexes does not alter mononuclear phagocyte system function.
Immunol; 48; 205-210.

Johnson P M et al. (1981)
Review article: Fc gamma receptors in the human placenta.
Placenta; 2; 355-370.

Johnson P M, Faulk W P (1976)
Rheumatoid factor: its nature, specificity and production in rheumatoid arthritis.
Clin Immunol Immunopathol; 6; 414-430.

Jones P A, Werb Z. (1980)
Degradation of connective tissue matrices by macrophages II. Influence of matrix composition on proteolysis of glycoproteins, elastin, and collagen by macrophages in culture.
J Exp Med; 152; 1527-1536.

Joseph M, Capron A, Butterworth A E, Sturrock R F, Houba V. (1978)
Cytotoxicity of human and baboon mononuclear phagocytes against schistosomula in vitro: induction by immune complexes containing IgE and Schistosoma mansoni antigens.
Clin Exp Immunol; 33; 48-56.

Kaplan G (1977).
Differences in the mode of phagocytosis with Fc and C3 receptors in macrophages.
Scand. J. Immunol; 6; 797-807.

Kaplan G, Eskeland T and Seljelid R (1978).
Difference in the effect of immobilised ligands on the Fc and C3 receptors of mouse peritoneal macrophages in vitro.
Scand. J. Immunol; 7; 19-24.

Kaplan M E, Jandl J H. (1961)
Inhibition of red cell sequestration by cortisone.
J Exp Med; 114; 921-937.

Karnovsky M L, Lazdin S J K, Simmons S R. (1975)
Metabolism of activated mononuclear phagocytes at rest and during phagocytosis.
In: van Furth ed; Mononuclear phagocytes in Immunity Infection and Pathology; pp423-439; Blackwell, Oxford.

Karnovsky M L, Simmons S, Glass E A, Shafer A W and D'Arcy P (1970).
Metabolism of macrophages.
In Mononuclear Phagocytes pp 103-120; (R van Furth ed; Blackwell Scientific Publications, Oxford).

Katayama S, Chia D, Nasu H, Knutson D W. (1981)
Increased Fc receptor activity in monocytes from patients with rheumatoid arthritis: A study of monocyte binding and catabolism of soluble aggregates of IgG in vitro.
J Immunol; 127; 643-647.

Katz D H and Unanue E R (1973).
Critical role of determinant presentation in the induction of specific responses in immuno-competent lymphocytes.
J. Exp. Med; 137; 967-990.

Kavai M, Lukacs K, Sonkoly I, Jokay I, Berndt A, Szegedi Gy. (1980)
Loss of monocyte membrane receptors in patients with SLE.
Clin Exp Immunol; 40; 66-71

Kavai M, Zsindely A, Sonkoly I, Major M, Demjan I, Szegedi Gy. (1983)
Signals of monocyte activation in patients with SLE.
Clin Exp Immunol; 51; 255-260.

Kavai M, Sandor M, Szegedi G Y, Fust G, Gergely J. (1981)
Effect of soluble immune complexes on Fc and C3 receptor dependent phagocytosis by human monocytes.
Immunology; 44; 599-606.

Kavai M, Lukacs K, Sonkoly I, Paloczi K, Szegedi G Y. (1979)
Circulating immune complexes and monocyte Fc function in autoimmune diseases.
Ann Rheum Dis; 38; 79-83.

Kawasaki T, Etoh R, Yamashina I. (1978)
Isolation and characterisation of a mannan binding protein from rabbit liver.
Biochem Biophys Res Commun; 81; 1018-1024.

Kay A B, Glass E J and Salter D McG (1979).
Leucoattractants enhance complement receptors on human phagocytic cells.
Clin. Exp. Immunol; 38; 294-299.

Kay M M B. (1975)
Mechanism of removal of senescent cell by human macrophages in situ.
Proc Nat Acad Sci USA; 72; 3521-3525.

Kazatchkine M D, Fearon D T, Appay M D, Mandet C, Bariety J. (1982)
Immunohistochemical study of the human glomerular C3b receptor in normal kidney and in 75 cases of renal disease.
J Clin Invest; 69; 900.

Kazatchkine M D, Fearon D T, Silbert J E and Austen K F (1979).
Surface-associated heparin inhibits zymosan-induced activation of the human alternative complement pathway by augmenting the regulatory action of the control proteins on particle-bound C3b.
J. Exp. Med; 150; 1202-1215.

Kelly J P, Johnson M C and Parker C W (1979).
Effect of inhibitors of arachidonic acid metabolism on mitogenesis in human lymphocytes: possible role of thromboxanes and products of the lipoxygenase pathway.
J. Immunol; 122; 1563-1571.

Kijlstra A, van der Lelij A, Knutson D W, Fleuren G J, Vanes L A. (1978)
The influence of phagocytic function on glomerular localisation of aggregated IgM in rats.
Clin Exp Immunol; 32; 207-217.

Kimberly R P, Ralph P. (1983)
Endocytosis by the mononuclear phagocyte system amd autoimmune disease.
Am J Med; 74; 481-493.

Kimberly P R, Parris T M, Inman R D, McDougal J S. (1983)
Dynamics of mononuclear phagocyte system Fc receptor function in SLE. Relation to disease activity and circulating immune complexes.
Clin Exp Immunol; 51; 261-268.

Kinsky R G, Christie G H, Elson J and Howard J G (1969).
Extra Hepatic deprivation of Kupffer cells during oestrogenic stimulation of parabiosed mice.
Br. J. Exp. Pathol; 50; 438.

Klaus G G B and Humphrey J H (1977).
The generation of memory cells. Role of C3 in the generation of B memory cells.
Immunol; 33; 31-40.

Klein G, Purtilo D. (1981)
Summary: Symposium on EB-virus induced lymphoproliferative diseases in immunodeficient patients.
Cancer Res; 41; 4302-4304.

Klein D C and Raisz L G (1970).
Prostaglandins: stimulation of bone resorption in tissue culture.
Endocrinology; 86; 1436.

van der Knaap W P et al. (1983)
Cytophilic receptors for foreignness and some factors which influence phagocytosis by invertebrate leucocytes: in vitro phagocytosis by amoebocytes of the snail Lymnea stagnalis.
Immunology; 48; 377-383.

Koffler D, Agnello V, Thoburn R, Kunkel H G. (1971)
Systemic lupus erythematosus: prototype of immune complex nephritis in man.
J. Exp. Med.; 134 (suppl); 169S-79S.

Koffler D, Biesecker G, Katz S M (1982)
Immunopathogenesis of systemic lupus erythematosus.
Arth & Rheum; 25; 858-861.

Koboyashi I and Ziff M (1975).
Electron microscopic studies of the cartilage-pannus junction in rheumatoid arthritis.
Arth Rheum; 18; 475.

Kolb H, Kolb-Bachofen V. (1978)
A lectin like receptor on mammalian macrophages.
Biochem Biophys Res Commun; 85; 678-683.

Kossard S, Nelson D S. (1968)
Studies on cytophilic antibodies IV. The effects of proteolytic enzymes (trypsin and papain) on the attachment to macrophages of cytophilic antibodies.
Aus J Exp Biol & Med Sci; 46; 63.

Kozel T R and Mastroianni R P (1976).
Inhibition of phagocytosis by cryptococcal polysaccharide: dissociation of the attachment and ingestion phases of phagocytosis.
Infect & Immun; 14; 62.

Kragballe K, Borregard N, Brandrup F, Koch C, Staehrjohansen K. (1981)
Relation of monocyte and neutrophil oxidative metabolism to skin and oral lesions in carriers of chronic granulomatous disease.
Clin Exp Immunol; 1981; 43; 390-8.

Krane S M (1981)
Aspects of the cell biology of the rheumatoid synovial lesion.
Ann Rheum Dis; 40; 433-448.

Kredich N M, Martin D W (Jr). (1977)
Role of S-adenosyl homocysteine in adenosine mediated toxicity in cultured mouse T-lymphoma cells.
Cell; 12; 931-938.

Kulczycki (Jr) A, Krause V, Killion C C and Atkinson J P (1980).
Purification of Fc-gamma receptor from rabbit alveolar macrophages that retains ligand-binding activity.
J. Immunol; 124; 2772-2779.

Kulka J P, Bocking D, Ropes M W, Bauer W. (1955)
Early joint lesions of rheumatoid arthritis. Report of eight cases, with knee biopsies of lesions of less than one year's duration.
Arch Pathol; 59; 129-50.

Kurland J I, Bockman R. (1978)
Prostaglandin E production by human blood monocytes and peritoneal macrophages.
J Exp Med; 147; 952-957.

Kurlander R J, Rosse W F and Logue G L. (1978)
Quantitative influence of antibody and complement coating of red cells on monocyte mediated cell lysis.
J Clin Invest; 61; 1309-1319.

Kurlander R J. (1981)
The effects of corticosteroids on IgG Fc receptor and complement receptor mediated interaction of monocyte with red cells.
Clin Immunol and Immunopath; 20; 325-335.

Lambris J D, Ross G D. (1981)
Characterisation of the lymphocyte membrane receptor for factor H (blH globulin) with an antibody to anti-factor H idiotype.
J Exp Med; 155; 1400-1411.

Lambris T D, Dobson N J, Ross G D. (1981)
Isolation of lymphocyte membrane complement receptor type 2 (the C3d receptor) and preparation of receptor specific antibody.
Proc Nat Acad Nat Sci USA; 78; 1828-1832.

Landry M.(1977)
Phagocyte function and cell mediated immunity in systemic lupus erythematosus.
Arch Dermatol; 113; 147-54.

Lane B C, Cooper S M. (1982)
Fc recptors of mouse cell lines. I. Distinct proteins mediate the IgG subclass specific Fc binding activities of macrophages.
J Immunol; 128; 1819-1824.

Lane B C, Kane Mitchell J, Mitchell M S, Cooper S M. (1980)
Structural evidence for distinct IgG subclass specific Fc receptors on mouse peritoneal macrophages.
J Exp Med; 152; 1147-1161.

Langevoort H L, Cohn Z A, Hirsch J G, Humphrey J N, Spector W G, van Furth R. (1970)
The nomenclature of mononuclear phagocytic cells. Proposal for a new classification.
In: van Furth R. ed; Mononuclear phagocytes; Blackwell, Oxford.

Lavie G, Zucher-Franklin, Franklin E C. (1980)
Elastase type proteases on the surface of human blood monocytes: possible role in amyloid formation.
J Immunol; 125; 175.

Law S K, Fearon D T, Levine R P. (1979)
Action of the C3b inactivator on cell bound C3b.
J Immunol; 122; 759-765.

Lawley T J et al. (1981)
Defective Fc receptor function associated with the HLA B8/DRW3 haplotype.
N Eng J Med; 304; 185-92.

Lay W H, Nussenzweig V. (1968)
Receptors for complement on leukocytes.
J Exp Med; 128; 991-1009.

Leclerc M, Panijel J, Redziniak G, Brillouet C, Binaghi R (1980)
Correlation between the injection of specific proteins in starfishes and the appearance of binding sites for the same proteins in cell populations of their axial organs.
Cell Immunol; 53; 1-9

Lee A. (1968)
Effect of neuraminidase on the phagocytosis of heterologous red cells by mouse peritoneal macrophages.
Proc Soc Exp Biol Med; 128; 891-894.

Leijh P C J, Van den Barselaar M T, van Furth R. (1977)
Kinetics of phagocytosis and killing of candida albicans by human granulocytes and monocytes.
Infec Immun; 17; 313-18.

Leijh P C J, van den Barselaar M Th, von Zwet T L, Daha M R, van Furth (1979)
Requirement of extracellular complement and immunoglobulin for intracellular killing of microorganisms by human monocytes.
J Clin Invest; 63; 772-784.

Lepow I H, Naff G B, Todd E W, Pensky J, Hinz C F (1963)
Chromatographic resolution of the first component of complement into three activities.
J Exp Med; 117; 983-1008

Lerner E A, Lerner M R, Hardin J A, Janeway Jr C A, Steitz J A. (1982)
Deciphering the mysteries of RNA-containing lupus antigens.
Arth & Rheum; 25; 761-766.

Lesavre P H, Muller-Eberhard H J (1978)
Mechanism of action of factor D of the alternative complement pathway.
J Exp Med; 148; 1498-1509

Leslie R G Q, Cohen S. (1974)
Cytophilic activity of IgG2 from sera of guinea pigs immunised with bovine gamma globulin.
Immunology; 27; 589-599.

Leslie R G Q, Cohen S. (1976)
Comparison of the cytophilic activities of guinea pig IgG1 and IgG2 antibidies.
Eur J Immunol; 6; 848-??.

Leslie R G Q, Alexander M D. (1979)
Cytophilic antibodies.
Cyrr Top Microbiol Immunol; 88; 25-104.

Leu R W, Herriot M J, Moore P E, Orr G R, Birckbichler P J. (1982)
Enhanced transglutaminase activity associated with macrophage activation. Possible role in Fc mediated phagocytosis.
Exp Cell Res; 141; 191-199.

Libman E, Sachs B. (1924)
A hitherto undescribed form of valvular and mural endocarditis.
Arch Int Med; 33; 701-737.

Littman B H, Ruddy S. (1977)
Production of the second component of complement by human monocytes: stimulation by antigen activated lymphocytes or lymphokines.
J Exp Med; 145; 1344-1352.

Lobuglio A F, Cotran R S, Jandl J H. (1967)
Red cells coated with immunoglobulin G: binding and sphering by mononuclear cells in man.
Science; 158; 1582.

Lockwood C M, Worlledge S, Nicholas A, Cotton C, Peters D K. (1979)
Reversal of impaired splenic function in patients with nephritis or vasculitis (or both) by plasma exchange.
N Eng J med; 300; 524-530.

Lohmann-Matthes M L, Domzig W, Roden J. (1979)
Promonocytes have the functional characteristics of natural killer cells.
J Immunol; 123; 1883-1886.

Lohmann-Matthes M L, Sun D M, Zahringer M. (1981)
Maturation of macrophages and macrophage mediated cytotoxicity.
In: Heterogeneity of mononuclear phagocytes.
Ed: Forster O, Landy M. Academic Press, Amsterdam.
486-495.

Loos M. (1982)
The functions of endogenous Clq a subcomponent of the first component of complement as a receptor on the membrane of macrophages.
Mol Immunol; 19; 1229-1238.

Loos H, Blok-Schut B, Kipp B, van Doorn R, Meerhof L.
Size distribution, electronic recognition and counting of human blood monocytes.
Blood; 1976; 48; 743-53.

Lunney J, Ashwell G. (1976)
A hepatic receptor of avian origin capable of binding specifically modified glycoproteins.
Proc Nat Acad Sci USA; 73;341-343.

McKeever P E, Garvin A J, Spicer S S. (1976)
Immune complex receptors on cell surfaces I. Ultrastructural demonstration on macrophages.
J Histochem Cytochem; 24; 948-1212.

McKenzie I FC, Clarke A, Parish C R. (1977)
Ia antigenic specificities are oligosaccharide in nature: hapten inhibition studies.
J Exp Med; 145; 1039-1053

Mackeness G B. (1962)
Cellular resistance to infection.
J Exp Med; 116; 381.

Mackeness G B. (1969)
The influence of immunologically committed lymphoid cells on macrophage activity in vivo.
J Exp Med; 129; 973.

McPherson G G, Steer H W. (1980)
The origin and properties of peripheral lymph mononuclear phagocytes.
In: van Furth, ed; Mononuclear phagocytes. Functional aspects Part I; pp299-317; Martinus Nijhoff, The Hague.

Malawista S E, Steere A S. (1981)
In: Kelley W N, Harris E D, Ruddy S, Sledge C B, eds; Textbook of Rheumatology; pp1586-1601; W B Saunders Company, Philadelphia.

Mann D L, Muchmore A. (1980)
Inhibition of DrW antisera reactivity by mono and oligosaccharides.
J Immunol; 124; 2218-2221.

Mannik M (1982)
Pathophysiology of circulating immune complexes.
Arth & Rheum; 25; 783-787.

Mannik M, Arend W P, Hall A P, Gilliland B C. (1971)
Studies on antigen-antibody complexes. I Elimination of soluble complexes from rabbit circulation.
J Exp Med; 133; 713-715.

Mantovani B, Rabinovitch M, Nussenzweig V. (1972)
Phagocytosis of immune complexes by macrophages. Different roles of the macrophage receptor sites for complement (C3) and for immunoglobulin (IgG).
J Exp Med; 135; 780-792.

Masucci M G, et al (1981)
Cellular immune defects to EB virus-determined antigens in young males.
Cancer Res; 41; 4282-4291.

Mattioli M, Reichlin M.
Characterisation of a soluble nuclear ribonucleoprotein antigen reactive with SLE sera.
J Immunol; 1971; 107; 1281-89.

Medicus R G, Chapius R M (1980)
The first component of component of complement. I. Purificatin and properties of native C1.
J Immunol; 125; 390-395

Medof M E, Iida K, Mold c, Nussenzweig V. (1982)
Unique role of the complement receptor (CR1) in the degradation of C3b associated with immune complexes.
J Exp Med; 156; 1739-1754

Melamed J, Arnaout M A, Dobler C, Colten H R. (1981)
Complement receptor ligand induced histaminase release from human granulocytes.
Fed Proc; 40; 1013 (abstr).

Melewicz F M, Boltz-Nitulesco G. Spiegelberg H L. (1981)
Receptors for IgE on human monocytes and rat macrophages.
In: Heterogeneity of mononuclear phagocytes;102-109.
Ed. Forster U, Landy M.
Acad Press; London, New York.

Mellman I R, Unkeless J C. (1980)
Purification of a functional mouse Fc receptor through the use of a monoclonal antibody.
J Exp Med; 152; 1048-1069.

Meruelo D, Edidin M. (1980)
The biological fucntion of the major histocompatibility complex: hypothesis.
In: Marchalonis J J, Cohen N, eds; Contempory topics in immunobiology; pp-231-253; Plenum Press, New York.

Meryhew N L, Runquist O A. (1981)
A kinetic analysis of immune mediated clearance of erythrocytes.
J Immunol; 126; 2443-2449.

Messner R P, Jelinek J. (1970)
Receptors for gamma globulin on human neutrophils.
J Clin Invest; 49; 2165-2171.

Metchnikoff E (1901)
L'Immunite dans les maladies infectieuses.
Masson; Paris.

Metzgar Z, Hoffield J T, Oppenheim J J. (1980)
Macrophage mediated suppression I. Evidence for participation of both H2O2 and prostaglandins in suppression of murine lymphocyte proliferation.
J Immunol; 124; 983-988.

Meuret G, Hoffman G. (1973)
Monocyte kinetic studies in normal and disease states.
B J Haem; 24; 275.

Michl J, Pieczonka M M, Unkeless J C, Silverstein S C. (1979)
Effects of immobilised immune complexes on Fc- and complement receptor function in resident and thioglycollate elicited mouse peritoneal macrophages.
J Exp Med; 150; 607-621.

Michl J, Ohlbaum D J, Silverstein S C. (1976)
2-deoxy glucose selectively inhibits Fc and complement receptor mediated phagocytosis in mouse peritoneal macrophages. II Dissociation of the inhibitory effects of 2-deoxy glucose on phagocytosis and ATP generation.
J Exp Med; 144; 1484.

Miescher P, Fauconnet M. (1954)
L'absorption du facteur "LE" par des noyaux cellulaires isoles.
Experientia; 10; 252-254.

Miller G W, Nussenzweig V. (1975)
A new complement function: solubilisation of antigen-antibody aggregates.
Proc Nat Acad Sci; 72; 418-422.

Miyakawa Y, Yamada A, Kosaka K.
Defective immune adherence (C3b) receptor on erythrocytes from patients with systemic lupus erythematosus.
Lancet; 1982; 493-97.

Mizel S B, Dayer J-M, Krane S M, Mergenhagen S E.
Stimulation of rheumatoid synovial cell collagenase and protaglandin production by partially purified lymphocyte activating factor (Interleukin I).
Proc. Natl. Acad. Sci. USA 1981; 78; 2474-7.

Moeller G. (Ed) (1981).
Role of Fc receptor bearing T-cells.
Immunol Rev; 56; (Munksgaard, Copenhagen).

Mohandas N, Phillips W M, Bessis M. (1979)
Red blood cell deformability and haemolytic anaemias.
Semin Haem;16; 95.

Mollerasmussen J, Brandslund I, Rasmussen G G, Svehag S E. (1982)
Increased numbers of IgG Fc receptors on monocyte enriched peripheral blood leucocytes from patients with RA.
Scan J Immunol; 16; 279-285.

Mollison P L. (1962)
The reticuloendothelial system and red cell destruction.
Proc Roy Soc Med; 55; 915.

Mongan E S, Cass R M, Jacox R F (1969)
A study of the relations of seronegative and seropositive rheumatoid arthritis to each other and to necrotising vasculitis.
Am J Med; 47; 23-35.

Morgan A G, Soothill J F (1975)
Relationship between macrophage clearance of PVP and affinity of antiprotein antibody response in inbred mouse strains.
Nature (London); 254; 711-712.

Morrison D C, Kline L F (1977)
Activation of the classical and properdin pathways of complement by bacterial liposaccharides (LPS).
J Immunol; 118; 362-368

Muchmore A V, Blaese R M. (1979)
Evidence that monocyte mediated cellular recognition phenomena are mediated by receptors with specificity for simple oligosaccharides.
Proceedings of the Brooke Lodge Symposium; 505-517.

Muller-Eberhard H J. (1975)
Complement.
Ann Rev Biochem; 44; 697-724.

Muller-Eberhard H J (1980)
Complement reaction pathways.
In: Fougereau M, Dausset J, eds; International Congress of Immunology, (4th) Paris.
Progress in Immunology IV.; ppl001-1024; Academic Press, New York.

Muller-Eberhard H J, Gotze O. (1972)
C3 proactivator convertase and its mode of action.
J Exp Med; 135; 1003-1008.

Mussel H H, Ehlen T, Schmitt M, Kazatchkine M D, Neyses L, Dierich M P. (1982)
Isolation and characterisation of the C3 binding entity from human erythrocytes.
Immun Lett; 4; 1-6.

Nagamura Y, Kolb H. (1981)
Rat peritoneal macrophages express two different lectin-like receptors on the cell surface.
In: Bog-Hansen T C, ed; Lectins - Biology, biochemistry, clinical biochemistry; vol 1; pp33-42; de Gruyter, Berlin, New York.

Nagamura Y, Kolb H. (1980)
Presence of a lectin like receptor for D-galactose on rat peritoneal macrophages.
FEBS letts; 115; 59-62.

Nagasawa S, Stroud R M. (1977)
Mechanism of action of the C3b inactivator requirement for a high molecular weight cofactor (C3b-C4bINA cofactor) and production of a new C3b derivative (C3b').
Immunochem; 14; 749

Nathan C F, Murray H W, Cohn Z A. (1980)
The macrophage as an effector cell.
N Eng J med; 303; 622-626.

Nelson D S. (1956)
The immune adherence phenomenon: a hypothetical role of erythrocytes in defence against bacteria and viruses.
Proc Roy Soc; 49; 55-58.

Nelson D S. (1963)
Immune adherence.
Adv Immunol; 3; 131-180.

Newman S L, Musson R A, Henson P M. (1980)
Development of functional complement receptors during in vitro maturation of human monocytes into macrophages.
J Immunol; 125; 2236-2244.

Newman S L, Johnston R B. (1979)
Role of binding through C3b and IgG in PMN function: studies with trypsin generated C3b.
J Immunol; 123; 1839-1846.

Ng K C, Brown K A, Perry J D, Holborow E J. (1980)
Anti-RANA antibody: a marker for seronegative and seropositive RA.
Lancet; 1; 447-449.

Nir S, Anderson M. (1977)
Van der Waals interactions between cell surfaces.
J Membrane Biol; 31;1

Nisonoff A, Wissler F C, Lipman L N, Woernley D L. (1960)
Separation of univalent fragments from the bivalent rabbit antibody by reduction of disulphide bonds.
Arch Biochem Biophys; 89; 230-244.

Nitta T, Suzuki T. (1982a)
Biochemical signals transmitted by Fc-gamma receptors: Triggering mechanisms of the increased synthesis of adenosine-3',5'-cyclic monophosphate mediated by Fc2a and Fc2b receptors of a murine macrophage-like cell line (P388D1).
J Immunol; 129; 2708-2717.

Nitta T, Suzuki T. (1982b)
Fc-gamma-2b receptor mediated prostaglandin synthesis by a murine macrophage cell line (P388D1)
J Immunol; 128; 2527.

Northcote D h, Horne R W. (1951)
The chemical composition and structure of the yeast cell wall.
Biochem J; 51; 232-236.

Norval M, Hart H, Marmion B P. (1979)
Viruses and lymphocytes in RA. I Studies on cultured rheumatoid lymphocytes.
Ann Rheum Dis; 38; 507-513.

Nussenzweig V. (1974)
Receptors for immune complexes on lymphocytes.
In: Adv in Immunology; 217-254.
Ed: Dixon F J, Kunkel H G.
Academic Press, New York.

Nydegger U N, Lambert P H. (1982)
Immune complexes and complement.
In: Panayi G S, ed; Scientific Basis of Rheumatology; pp74-86;
Churchill Livingstone.

Ofek I, Mirelman D, Sharon N. (1977)
Adherence of E. coli to human mucosal cells mediated by mannose receptors.
Nature; 265; 623.

Ogmundsdottir H M, Weir D M. (1976)
The characteristics of binding of Corynebacterium parvum to glass adherent mouse peritoneal exudate cells.
Clin Exp Immunol; 26; 334-339.

Ogmundsdottir H M, Weir D M, Marmion B P. (1978)
Binding of microorganisms to the macrophage plasma membrane, effects of enzymes and periodate.
B J Exp Path; 59; 1-7.

Ogmundsdottir H M, Weir D M. (1980)
Mechanisms of macrophage activation.
Clin Exp Immunol; 40; 223.

Ogmundsdottir H M, Weir D M. (1979)
Stimulation of phosphatidyl inositol turnover in the macrophage plasma membrane: a possible mechanism for signal transmission.
Immunology; 37; 689-696

Oldstone M B, Dixon F J. (1972)
Inhibition of antibodies to nuclear antigen and to DNA in New Zealand mice infected with lactate dehydrogenase virus.
Science; 175; 784-793.

Oldstone M B, Dixon F J. (1969)
Pathogenesis of chronic disease associated with lymphocyte choriomeningitis viral infection. I. Relationship of antibody production to disease in neonatally infected mice.
J Exp Med; 129; 483-505.

Oliver A and Weir D M (1983)
Inhibition of bacterial binding to mouse macrophages by pseudomonas alginate.
J Lab Clin Immunol; 10; 221-224.

Osler W (1904)
On the visceral manifestations of the erythema group of skin diseases.
Am J Med Sci; 127; 1-23.

Panayi G S. (1982)
In Panayi G S, ed; Scientific Basis of Rheumatology; p58; Churchill Livingstone.

Panayi G S, Wooley P H, Batchelor J R. (1978)
Genetic basis of rheumatoid disease: HLA antigens, disease manifestations and toxic reactions to drugs.
B M J; 2; 1326-1328.

Pangburn M K, Schreiber R D, Muller-Eberhard. (1977)
Human complement C3b inactivator: isolation, characterisation and demonstration of an absolute requirement for the serum protein blH for cleavage of C3b and C4b in solution.
J Exp Med; 146; 257.

Papadimitriou J M. (1973)
Detection of macrophage receptors for heterologous IgG by scanning and transmission electron microscopy.
J Pathol; 110; 213.

Pappas M G, Nussenzweig R S, Nussenzweig V, Shear H L. (1981)
Complement mediated defect in clearance and sequestration of autologous erythrocytes in rodent malaria.
J Clin Invest; 67; 183.

Parish C R, Chilcott A B, McKenzie I F C. (1976a)
Low molecular weight Ia antigens in normal mouse serum.
I. Detection and production of a xenogeneic antiserum.
Immunogenetics; 3; 113-128.

Parish C R, Jackson D C, McKenzie I F C. (1976b)
Low molecular weight Ia antigens in normal mouse serum.
III. Isolation and partial chemical characterisation.
Immunogenetics; 3; 455-463.

Parish C R, Higgins T J, McKenzie I F C. (1978).
Comparison of antigens recognised by xenogeneic and allogeneic anti-Ia antibodies: evidence for two classes of Ia antigens.
Immunogenetics; 6; 343-354.

Parrish T M, Kimberly R P, Inman R D, McDougal J S, Gibofsky A, Christian C L. (1982)
Defective Fc receptor mediated function of the mononuclear phagocyte system in lupus nephritis.
Ann Int Med; 97; 526-532.

Passwell J H, Dayer J-M, Merler E. (1979)
Increased prostaglandin production by human monocytes after membrane receptor activation.
J. Immunol; 123; 115-20.

Passwell J H, Dayer J M, Gass K, Edelson P J. (1980)
Regulation by Fc fragments of the secretion of collagenase, PGE2, and lysozyme by mouse peritoneal macrophages.
J Immunol; 125; 910-913.

Passwell J H, Steward M W, Soothill J F (1974)
Intermouse strain differences in macrophage function and its relationship to antibody response.
Clin Exp Immunol; 17; 159-167.

Paty J G (Jr), Sienknecht C W, Townes A S, et al.(1975)
Impaired cell mediated immunity in systemic lupus erythematosus.
Am J Med; 59; 769-79.

Pearse B M F. (1975)
Coated vesicles from pig brain: purification and biochemical characterisation.
J Mol Biol; 97; 93.

Pepys M B. (1976)
Role of complement in the induction of immunological responses.
Transplant. Rev; 32; 93-120.

Petty H R, Smith L M, Fearon D T, McConnell H M. (1981)
Lateral distribution and diffusion of the C3b receptor of complement, HLA antigens, and lipid probes in peripheral blood leucocytes.
Proc Nat Acad Sci USA; 77; 6587-6591.

Pfueller S L, Weber S, Luscher E F. (1977)
Studies of the mechanism of the human platelet release reaction induced by immunologic stimuli III. Relationship between the binding of soluble IgG aggregates to the Fc receptor and cell response in the presence and absence of plasma.
J Immunol; 118; 514-524.

Phillips P E, Lim W N, Parkman P D, Harshaut Y. (1973)
Virus antibody and IgG levels in Juvenile Rheumatoid Arthritis.
Arth & Rheum; 16; 126.

Pick E, Mizel D. (1982)
Role of transmethylation in the elicitation of an oxidative burst in macrophages.
Cell Immunol; 72; 277-285.

Pike M C, Snyderman R. (1982)
Transmethylation reactions regulate affinity and functional activity of chemotactic factor receptors on macrophages.
Cell; 28; 107-114.

Pillemer L, Blum L, Lepow I H, Ross O A, Todd E W, Wardlaw A C (1954)
The properdin system and immunity: I demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena.
Science; 120; 279.

Porter R R, Reid K B M. (1979)
Activation of the complement system by antigen-antibody complexes: The classical pathway.
Adv Protein Chem; 33; 1-71.

Pincus T (1982)
Studies regarding a possible function for viruses in the pathogenesis of systemic lupus erythematosus.
Arth & Rheum; 25; 847-856.

Postlethwaite A E, Lachman L B, Mainardi C L, Kang A H. (1983)
Interleukin 1 stimulation of collagenase production by cultured fibroblasts.
J Exp Med; 157; 801-806.

Pritchard D G, Settine R L, Bennett J C. (1980)
Sensitive mass spectrometric procedure for the detection of bacterial cell wall components in rheumatoid joints.
Arth & Rheum; 23; 608-610.

Pudliachouk L, Eyquem A, Jacqueline F. (1958)
Le diagnostic de la polyarthrite chronique evolutive par agglutination des globules rouge humain sensibiles.
Ann de Inst Pasteur; 94; 659.

Rabinovitch M, Manejias R E, Nussenzweig V. (1975)
Selective phagocytic paralysis induced by immobilised immune complexes.
J Exp Med; 142; 827-838.

Raepple E, Hill H-U, Loos M (1976)
Mode of interaction of different polyanions with the first (C1,C1), the second (C2) and the fourth (C4) component of complement.
Immunochem; 13; 251-255

Ragsdale C G, Arend W P. (1980)
Loss of Fc receptor activity after culture on surface-bound immune complexes. Mediation by cyclic nucleotides.
J Exp Med; 151; 32-44.

Raij L, Sibley R K, Keane W F. (1981)
Mononuclear phagocytic system stimulation. Protective role from glomerular immune complex deposition.
J Lab Clin Med; 98; 558-567.

Ralph P, Ito M, Broxmeyer H E, Nakoinz I. (1978)
Corticosteroids block newly induced but not constitutive functions of macrophage cell lines: myeloid colony stimulating activity production, latex phagocytosis and antibody dependent lysis of erythrocytes and tumour targets.
J Immunol; 121; 300-303.

Ralph P, Nakoinz I, Diamond B, Yelton D. (1980)
All classes of murine IgG antibody mediate macrophage phagocytosis and lysis of erythrocytes.
J Immuol; 125; 1885-1888.

Razin E, Bauminger S, Globerson A. (1978)
Effect of prostaglandins on phagocytosis of sheep erythrocytes by mouse peritoneal macrophages.
J Retic Soc; 23; 237.

Remold H G. (1973).
Requirement for alpha-L-fucose on the macrophage membrane receptor for MIF.
J Exp Med; 138; 1065-1076.

Reynolds H Y, Atkinson J P, Newball H H, Frank M M. (1975)
Receptors for immunoglobulin and complement on human alveolar macrophages.
J Immunol; 114; 1813.

Reynolds H Y. (1974)
Pulmonary host defenses in rabbits after immunisation with Pseudomonas antigens: the interaction of bacteria, antibodies, macrophages and lymphocytes.
J Infec Dis; 130s; s134-s142.

Rickinson A B, et al (1981)
Long term T-cell mediated immunity to EB virus.
Cancer Res; 41; 4216-4221.

Rinehardt J J, Sagone A L, Balcerzak S P, Ackerman G A, Lobuglio A F.(1975)
Effects of corticosteroid therapy on human monocyte function.
N Eng J Med; ??; 236-41.

Rinehardt J J, Balcerzak S P, Sagone A L, Lobuglio A F. (1974)
Effect of corticosteroids on human monocyte function.
J Clin Invest; 54; 1337-1343.

Rivero I, Abaca H E, Valles R, Vannucci J D, Diumenjo M S,
Moravenik M B (1979)
A simple method to detect complement receptors using bakers yeast:
YC rosettes.
Scan J Immunol; 9; 9-14.

Robineaux R, Pinet J (1960)
An in vitro study of some mechanisms of antigen uptake by cells.
In CIBA Foundation Symposium on Cellular Aspects of Immunity;
pp5-40; Chuechill, London.

Rodnan G P, Schumacher H R. eds (1983)
Primer on the rheumatic diseases; 8th edition; pp30-33; Arthritis
Foundation, Atlanta, Georgis.

Ropes M W, Bennett G A, Cobb S, Jacox R, Jessar R A. (1959)
Diagnostic criteria for rheumatoid arthritis. 1958 revision.
Ann Rheum Dis; 18; 49-53.

Rose H M, Ragan C, Pearce E, Lipman M O. (1948)
Differential agglutination of normal and sensitised sheep
erythrocytes by sera of patients with rheumatoid arthritis.
Proc Soc Exp Biol Med; 68; 1-6

Rosenthal A S, Barcinski M A, Blake J T. (1977)
Determinant selection is a macrophage dependent immune response
gene function.
Nature; 267; 156.

Rosenthal C J, Franklin E C. (1975)
Depression of cellular mediated immunity in systemic lupus
erythematosus. Relation to disease activity.
Arth & Rheum; 18; 207-17.

Ross G D and Polley M J.
Specificity of human lymphocyte complement receptors.
J Exp Med; 1975; 141; 1163-1180.

Ross G D. (1980)
Analysis of the different types of leucocyte membrane complement receptors and their interaction with the complement system.
J Immunol Meth; 37; 197

Ross G D, Jarowski C I, Rabelino E M, Winchester R J. (1978)
The sequential appearance of Ia like antigens and two different complement receptors during the maturation of human neutrophils.
J Exp Med; 147; 730.

Ross G D, Lambris J D. (1982)
Identification of a C3bi specific membrane complement receptor that is expressed on lymphocytes, monocytes, neutrophils and erythrocytes.
J Exp Med; 155; 96-110.

Ross G D, Rabellino E M. (1979)
Identification of a neutrophil and monocyte complement receptor (CR3) that is distinct from lymphocyte CR1 & CR2 and specific for a site contained within C3bi.
Fed Proc; 38; 1467.

Ross G D, Polley M J, Rabellino E M, Grey H M. (1973)
Two different complement receptors on human lymphocytes - one specific for C3b and one specific for C3b inactivator cleaved C3b.
J Exp Med; 138; 798-811.

Rosse W F. (1968)
Fixation of the first component of complement by human antibodies.
J Clin Invest; 47; 2430-2445.

Rothman J E, Lenard J. (1977)
Membrane asymmetry.
Science; 195; 743-753

Rouzer C A, Scott W A, Kempe J, Cohn Z A. (1980a)
Prostaglandin synthesis by macrophages requires a specific receptor-ligand interaction.
Proc Nat Acad Sci USA; 77; 4279-4282.

Rouzer C A, Scott W A, Cohn Z A, Blackburn P, Manning J M. (1980b)
Mouse peritoneal macrophages release leukotriene C in response to a phagocytic stimulus.
Proc Nat Acad Sci USA; 77; 4928-4932.

Rouzer C A, Scott W A, Hamill A L, Liu Fu-Tong, Katz D H, Cohn Z A. (1982)
IgE immune complexes stimulate arachadonic acid release by mouse peritoneal macrophages.
Proc Nat Acad Sci USA; 79; 5656-5660.

Ruddy S, Austen K F. (1971)
C3b inactivator of man. II Fragments produced by C3b inactivator cleavage of cell-bound or fluid phase C3b.
J Immunol; 107; 742-750.

Ruddy S, Colten H R. (1974)
Rheumatoid arthritis. Biosynthesis of complement proteins by synovial tissues.
N Eng J Med; 290; 1284.

Rutherford B, Schenkein H A. (1983)
C3 cleavage products stimulate release of prostaglandins by human mononuclear phagocytes in vitro.
J Immunol; 130; 874-877.

Ryter A, DeChastelier C. (1977)
Morphometric and cytochemical studies of diclyostelium discoideum in regulative phase.
J Cell Biol; 75; 200

Saba T M, Jaffe M (1980)
Plasma fibrinonectin (opsonic glycoprotein): its synthesis by vascular endothelial cells and role in cardiopulmonary integrity after trauma as related to reticuloendothelial funcion.
Am J Med; 68; 577-594.

Saklatvala J. and Dingle J T. (1980)
Identification of catabolin, a protein from synovium which induces degradation of cartilage in organ culture.
Buichem Biophys Res Commun; 96; 1225;

Salky N K, Mills D, DiLuzio N R. (1965)
Activity of the reticuloendothelial system in diseases of altered immunity.
J Lab Clin Med; 66; 952-960.

Salmon J, Kimberly R P. (1983)
Defective mononuclear phagocyte function in vivo and in vitro in SLE.
Arth & Rheum; 26; S36

Schaller J. (1972)
Illness resembling lupus erythematosus in mothers of boys with chronic granulomatous disease.
Ann Intern Med; 76; 747-750.

Sched E S, Inman R D, Parris T M, Kimberly R P, Redecha P B, Christian C L. (1982)
Serial circulating immune complexes and reticuloendothelial system Fc receptor function in infectious endocarditis.
Arth & Rheum; 24; S74.

Schlepper-Schaffer J, Friedrich E, Kolb H. (1981)
Galactosyl specific receptor on liver cells. Binding site for tumour cells on both Kupffer cells and hepatocytes.
Eur J Cel Biol; 25; 95-102.

Schlepper-Schaffer J, et al (1980)
Rat liver Kupffer cells bind neuraminidase treated rat erythrocytes via galactose specific receptors.
Biochem J; 186; 827-831.

Schlesinger P H, Doebeer T W, Mandell B F, White R, de Schryver C, Rodman J S, Miller M J, Stahl P. (1978)
Plasma clearance of glycoproteins with terminal mannose and N-acetylglucosamine by liver non-parenchymal cells.
Biochem J; 176; 103-109.

Schlesinger P H, Rodman J S, Doebber T W, Stahl P D, Lee Y C, Stowell C P, Kuhlenschmidt T B. (1980)
The role of extrahepatic tissues in the receptor mediated plasma clearance of glycoproteins terminated by mannose or N-acetylglucosamine.
Biochem J; 192; 597-606.

Schlesinger P H, Rodman J S, Frey M, Lang S, Stahl P. (1976)
Clearance of lysosomal hydrolases following intravenous infusion.
Arch Biochem Biophys; 177; 606-614.

Schmidt M E, Douglas S D. (1976)
Effects of levamisole on human monocyte function and immunoprotein receptors.
Clin Immunol Immunopathol; 6; 299-305.

Schmitt M, Mussel H H, Dierich M P. (1981b).
Qualitative and quantitative assessment of C3 receptor reactivities on lymphoid and phagocytic cells.
J Immunol; 126; 2042-2047.

Schmitt M, Mussel H H, Hammann K P, Scheiner O, Dierich M P. (1981a)
Role of blH for the binding of C3b coated particles to human lymphoid and phagocytic cells.
Eur J Immunol; 11; 739:745.

Schneider R J, Kulczycki A (Jr), Law S K, Atkinson J P. (1981)
Isolation of a biologically active macrophage receptor for the third component of complement.
Nature; 290; 789-792.

Schneider R J, Atkinson J P, Krause V, Kulczycki A. (1981)
Characterisation of ligand binding activity of isolated murine Fc-gamma receptor.
J Immunol; 126; 735-740.

Schopf R E, Hamman K P, Scheiner O, Lemmel E M, Dierich M P. (1982)
Activation of both human monocytes by both blH and C3b.
Immunology; 46; 307-312.

Schooley R T et al (1983)
Association of Herpes virus infection with T-lymphocyte subset alterations, glomerulopathy, and opportunistic infections after renal transplantation.
N Eng J Med; 308; 307-313.

Schreiber L, Penny R. (1979)
Tissue C3b receptors.
Clin Exp Immunol; 38; 316-322.

Schreiber A D, Parsons J, McDermott P, Cooper R A. (1975)
Effect of corticosteroids on the human monocyte IgG and complement receptors.
J Clin Invest; 56; 1189-1197.

Schreiber R D, Pangburn M K, Bjornson A B, Brothers M A, Muller-Eberhard J H. (1982).
The role of C3 fragments in endocytosis and extracellular cytotoxic reactions by polymorphonuclear leucocytes.
Clin Immunol Immunopathol; 23; 335-357.

Schorlemmer H V, Sedlacek H H, Seiler F R, Bitter-Suerman D. (1981)
Evidence for the participation of endogenous C3 in macrophage stimulation.
In: Forster O, Landy M, eds; Heterogeneity of mononuclear phagocytes; pp298-300; Academic Press, London, New York.

Schorlemmer H V, Bitter-Suermann D, Allison A C. (1977)
Complement activation by the alternative pathway and macrophage enzyme secretion in the pathogenesis of chronic inflammation.
Immunology; 32; 929.

Schorlemmer H V, Allison A C. (1976)
Effects of activated complement components on enzyme secretion by macrophages.
Immunology; 31; 781

Schorlemmer H V, Davies P, Allison A C. (1976)
Ability of activated complement components to induce lysosomal enzyme release from macrophages.
Nature (London); 261; 48.

Schur P H. (1982)
Complement and lupus erythematosus.
Arth & Rheum; 25; 793-798.

Schwartz R H, Dickler H B, Sachs D H, Schwartz B D. (1976)
Studies of Ia antigens on murine peritoneal macrophages.
Scand J Immunol; 5; 731.

Scott W A, Zrike J M, Hamill A L, Kempe J, Cohn Z A. (1980)
Regulation of arachidonic acid metabolism in macrophages.
J Exp Med; 152; 324-335.

Segal D M, Horwitz E. (1977)
Binding of affinity cross-linked oligomers of IgG to cells bearing Fc receptors.
J Immunol; 118; 1338

Segal D M, Sharrow S O, Jones J F, Siraganian R P. (1981)
Fc (IgG) receptors on rat basophilic leukaemia cells.
J Immunol; 126; 138-145.

Segal D M, Titus T A. (1978)
The subclass specificity for the binding of murine myeloma proteins to macrophage and lymphocyte cell lines and to normal spleen cells.
J Immunol; 120; 1395

Segerling M, Opferkuch W. (1977)
C5 dependent enhancement of rosette formation and phagocytosis by human polymorphonuclear leucocytes.
Monogr Allergy; 12; 101-104.

Seitz M, Deimann W, Gram N, Hunstein W, Gemsa D. (1982)
Characterisation of blood mononuclear cells of rheumatoid arthritis patients I. Depressed lymphocyte proliferation and enhanced prostanoid release from monocytes.
Clin Immunol Immunopathol; 25; 405-416.

Sergent J S, Lockshin M D, Christian C L, Gocke D J. (1976)
Vasculitis with hepatitis B antigenaemia: long term observations in 9 patients.
Medicine; 55; 1-18.

Shaw D R, Griffin F M (Jr). (1981)
Phagocytosis requires repeated triggering of macrophage phagocytic receptors during particle ingestion.
Nature; 289; 409-411.

Shaw G M, Levy P C. (1978)
Human monocyte cytotoxicity to tumour cells I. Antibody dependent cytotoxicity.
J Immunol; 121; 573-578.

Shepherd V L, Lee Y C, Schlesinger P H, Stahl P. (1981)
L-fucose terminated glycoconjugates are recognised by pinocytosis receptors on macrophages.
Proc Nat Acad Sci USA; 78; 1019-1022.

Shoenfeld Y et al (1983)
Polyspecificity of monoclonal lupus autoantibodies produced by human-human hybridomas.
N Eng J Med; 308; 414-420.

Short C L. (1959)
Rheumatoid Arthritis: historical aspects.
J Chron Dis; 10; 367-387.

Shur B D, Roth S. (1975)
Cell surface glycosyl transferases.
Biochim Biophys Acta; 415; 473.

Silverstein S C, Loike J D (1980)
Phagocytosis.
In: van Furth ed; Mononuclear phagocytes. Functional aspects Part II; pp895-917; Martinus Nijhoff, The Hague.

Silverman S, Schumacher H R. (1981)
Antibodies to Epstein Barr viral antigens in early Rheumatoid Arthritis.
Arth & Rheum; 24; 1465-1468.

Singer S J, Nicolson G L. (1972)
The fluid mosaic model of the structure of cell membranes.
Science; 175; 720-731

Slaughter L, Carson D A, Jensen F C, Holbrook T L, Vaughan J H. (1978)
In vitro effects of EB virus on peripheral blood mononuclear cells from patients with RA and normal subjects.
J Exp Med; 148; 1429-1434.

Smolen J E, Weissman G. (1980)
Effects of indomethacin, 5,8,11,14-eicosatetraenoic acid para-phenacyl bromide on lysosomal enzyme release and superoxide anion generation by human polymorphonuclear leucocytes.
Biochem Pharmacol; 29; 533-538.

Snell G D. (1978)
T cells, T cell recognition structures and the major histocompatibility complex.
Immunol Rev; 38; 3-69.

van Snick J L, Masson P L. (1978)
The effect of complement on the ingestion of soluble antigen-antibody complexes and IgM aggregates by mouse peritoneal macrophages.
J Exp Med; 148; 903-914.

Sonderstrop G, Rubin B, Sorenson S F, Svejgaard A. (1978)
Importance of HLA-D antigens for the cooperation between human monocytes and T-lymphocytes.
Eur J Immunol; 8; 520.

Soothill J F, Steward M W. (1971)
The immunopathological significance of the heterogeneity of antibody affinity.
Clin Exp Immunol; 9; 193-199.

Sorg C. (1982)
Heterogeneity of macrophages in response to lymphokines and other signals.
Mol Immunol; 19; 1275-1278.

Stahl P D, Schlesinger P H. (1980)
Receptor mediated pinocytosis of mannose/N-acetylglucosamine terminated glycoproteins and lysosomal enzymes by macrophages.
Trends in biochemical science; 194;???

Stahl P, Schlesinger P H, Sigardson E, Rodman J S, Lee Y C. (1980)
Receptor mediated pinocytosis of mannose glyconjugates by macrophages: characterisation and evidence for receptor recycling.
Cell; 19; 207-215.

Stahl P D, Rodman J S, Miller M J, Schlesinger P H. (1978)
Evidence for receptor mediated binding of glycoproteins, glycoconjugates and lysosomal glycosidases by alveolar macrophages.
Proc Nat Acad Sci USA; 75; 1399-1403

Staples P J, Gerding D N, Decker J L and Gordon R S. (1974)
Incidence of infection in sytemic lupus erythematosus.
Arth & Rheum; 17; 1-10.

Stastny P (1980)
Joint report on RA.
In: Terasaki P I, ed; Histocompatibility testing; pp681-686; UCLA Tissue Typing Laboratory Press, Los Angeles.

Steere A C, et al (1979)
Chronic Lyme arthritis: clinical and immunogenetic differentiation from rheumatoid arthritis.
Ann Int Med; 90; 896-901.

Steere A C, et al (1980)
Elevated levels of collagenase and prostaglandin E2 from synovium associated with erosion of cartilage and bone in a patient with chronic Lyme arthritis.
Arth & Rheum; 23; 591-599.

Steere A C, et al (1983)
The spirochaetal aetiology of Lyme disease.
N Eng J Med; 308; 733-740.

Steinberg A D, Smathers P A, Boegel W. (1981)
Effects of sex hormones on autoantibody production by NZB mice and modification by environmental factors.
Clin Immunol Immunopathol; 17; 562-572.

Steinman R M, Brodie S E, Cohn Z A. (1976)
Membrane flow during pinocytosis. A stereological analysis.
J Cell Biol; 68; 665-687.

Steinman R M, Cohn Z A. (1972)
The interaction of a particulate horseradish peroxidase (HRP)-anti(HRP) immune complexes with mouse peritoneal macrophages in vitro.
J cell Biol; 155; 616.

Stendahl O I, Hartwig J H, Brotschi E A, Stossel T P. (1980).
Distribution of actin-binding protein and myosin in macrophages during spreading and phagocytosis.
J Cell Biol; 84; 215-224.

Steward M W, Gage S E, Petty R E. (1974)
Low affinity antibody production in mice - a form of immunological tolerance?
Eur J Immunol; 4; 751-757.

Steward M W, Devey M E. (1981)
Antigen-antibody complexes: their nature and role in animal models of antigen-antibody complex disease.
In: Immunological aspects of rheumatology.
Ed: W. Carson Dick.
63-91.

Stewart J, Glass E J, Weir D M. (1982)
Macrophage binding of S. albus is blocked by anti-I region alloantibody.
Nature; 298; 852-854.

Stockert R, Morell A, Scheinberg I (1976)
The existence of a second route for the transfer of certain glycoproteins from the circulation into the liver.
Biochim Biophys Res Comm; 68; 988-993

Stossel T P, Murrad F, Mason R J, Vaughan M. (1970)
Regulation of glycogen metabolism in polymorphonuclear leucocytes.
J Cell Biol; 245; 6228.

Stossel T P, Hartwig J H. (1976)
Interactions of actin, myosin, and a new actin-binding protein of rabbit pulmonary macrophages. II Role in cytoplasmic movement and phagocytosis.
J Cell Biol; 68; 602-619.

Stossel T P, Mason R J, Smith A L (1974)
Lipid peroxidation by human blood phagocytes.
J Clin Invest; 54; 638-645.

Sung S J, Nelson R S, Silvertstein S C. (1983)
Yeast mannans inhibit binding and phagocytosis of zymosan by mouse peritoneal macrophages.
J Cell Biol; 96; 160-166.

Suzuki T, Saito-Taki T, Radisavan R, Nitta T. (1982)
Biochemical signal transmitted by Fc gamma receptors: phospholipase A2 activity of Fc-gamma-2b receptor of murine cell line P388D1.
Proc Nat Acad Sci USA; 79; 591-595.

Svensson B, Norberg R, Torstensson R. (1980)
Effects of cryoglobulins and aggregated IgG on in vitro monocyte phagocytosis.
Scand J Rheum; Suppl 31; 57-65.

Svensson B, Hedburg H. (1973)
Impaired phagocytosis by macrophages in SLE.
Scand J Rheumatol; 2; 78-80.

Svensson B. (1975)
Serum factors causing impaired macrophage function in SLE.
Scand J Immunol; 4; 145-150.

Svensson B.(1980a)
Monocyte in vitro function in systemic lupus erythematosus I. A clinical and immunological study.
Scand J Rheum; Suppl 31; 29-41.

Svensson B. (1980b)
Occurrence of deficient monocyte yeast cell phagocytosis in presence of rheumatic sera.
Scand J Rheum; Suppl 31; 21-27.

Tack B F, Janatova J, Lorenz P E, Schechter A N, Prahl J W. (1980)
The third component of human complement. Appearance of a sulphydryl group following chemical or enzymatic inactivation.
J Immunol; 124; 1542.

Taylor J C, Crawford I P, Hugli T E. (1977)
Limited degradation of the third component (C3) of human complement by human leucocyte elastase: partial characterisation of C3 fragments.
Biochemistry; 16; 575

Temple A, Loewi G. (1977)
The effect of sera from patients with connective tissue diseases on red cell binding and phagocytosis by monocytes.
Immunology; 33; 109.

Tenner A J, Cooper N R. (1981)
Identification of types of cells in human peripheral blood which bind C1q.
J Immunol; 126; 1174-1179.

Tenner A J, Cooper N R. (1982)
Stimulation of a human polymorphonuclear leucocyte oxidative response by the Clq subunit of the first complement component.
J Immunol; 128; 2547-2552.

Tenner A J, Cooper N R. (1980)
Analysis of receptor mediated Clq binding to human peripheral blood mononuclear cells.
J Immunol; 125; 1658

Territo M C, Cline M J. (1977)
Monocyte function in man.
J Immunol; 118; 187-192.

Theofilopoulos A N, Carson D A, Tavaloni M, Slovin S F, Speers W C, Jensen F B, Vaughan J H. (1980)
Evidence for the presence of receptors for C3 and IgG on human synovial cells.
Arth Rheum; 23; 1-9.

Thompson E N and Soothill J F (1970)
Chronic granulomatous disease: quantitative clinicopathological relationships.
Arch Dis Child; 45; 24-32.

Tosato G, Magrath I T, Blaese R M. (1982)
T-cell mediated immunoregulation of EB virus-induced B lymphocyte activation in EBV-seropositive and EBV-seronegative individuals.
J Immunol; 128; 575-579.

Tosato G, Steinberg A D, Blaese R M. (1981)
Defective EBV specific suppressor T-cell function in rheumatoid arthritis.
N Eng J Med; 305; 1238-1243.

Townsend R, Stahl P. (1981)
Isolation and characterisation of a mannose/N-acetylglucosamine/fucose binding protein from rat liver.
Biochem J; 194; 209-214.

Unanue E R, Calderon J. (1975)
Evaluation of the role of macrophages in immune induction.
Fed Proc; 34; 1737-42.

Unanue E R. (1976)
Secretory function of mononuclear phagocytes.
Am J Path; 83; 396-417.

Unkeless J C, Kaplan G, Plutner H, Cohn Z A. (1978)
Fc receptor variants of a macrophage cell line.
Proc Nat Acad Sci USA; 76; 1440

Unkeless J C. (1977)
The presence of two Fc receptors on mouse macrophages: Evidence from a variant cell line and differential trypsin sensitivity.
J Exp Med; 145; 931

Unkeless J C. (1979)
Characterisation of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors.
J Exp Med; 150; 580-596.

Unkeless J C, Eisen H N. (1975)
Binding of monomeric immunoglobulins to Fc receptors of mouse macrophages.
J Exp Med; 142; 1520-1533

Unkeless J C, Gordon S, Reiche E J. (1974)
Secretion of plasminogen activator by stimulated macrophages.
J Exp Med; 139; 834-850.

Valone F H, Franklin M, Sun F F, Goetzl E J. (1980)
Alveolar macrophage lipoxygenase products of arachidonic acid: isolation and recognition as the predominat constituents of the neutrophil chemotactic activity elaborated by alveolar macrophages.
Cell Immunol; 54; 390-401.

Vaughan R B. (1965)
The discriminative behaviour of rabbit phagocytes.
B J Exp Path; 46; 71

Venables P J W, Roffe L M, Erhardt C C, Maini R N, Edwards J M B, Porter A D. (1981)
Titres of antibody to RANA in rheumatoid arthritis and normal sera.
Arth & Rheum; 24; 1459-1464.

Venables P J W, Edwards J M B, Maini R N. (1980)
Antibody to RANA and other nuclear antigens in infectious mononucleosis and rheumatoid arthritis.
Ann Rheum Dis; 39; 191.

Vernon-Roberts B, Jessop J D, Dore J. (1973)
Effects of gold salts and prednisolone on inflammatory cells. II Suppression of inflammation and phagocytosis in the rat.
Ann Rheum Dis; 32; 301-307.

Vogt W, Schmidt G, von Buttlar B, Diemenger L. (1978)
A new function of the activated third component of complement binding to C5, an essential step for C5 activation.
Immunol; 34; 29-40.

Vose B M, Harding M, White W, Moore M, Gallagher J. (1983)
Effect of simple sugars on natural killing: evidence against the involvement of a lectin-like mechanism in target recognition.
Clin Exp Immunol; 51; 517-524.

Vranian G, Conrad D, Ruddy S. (1980)
C3 mediates phagocytosis by rat peritoneal mast cells.
J Immunol; 124; 1544.

van Waarde D, Hulsing-Hesselink E, van Furth R. (1975)
Humoral regulation of monocytosis during an acute inflammatory reaction.
In: van Furth ed; Mononuclear phagocytes in immunity, infection and pathology; pp205-222.

van Waarde D, Hulsing-Hesselink E, van Furth (1978)
Humoral control of monocytopoesis by an activator and an inhibitor.
Agents & Actions; 8; 432.

Wahl S M, Mergenhagen S E, Martin G R. (1974)
Collagenase production by endotoxin activated macrophages.
Proc Nat Acad Sci; 72; 3598.

Walker W S. (1976)
Separate Fc receptors for immunoglobulins IgG2a and IgG2b on an established cell line of mouse macrophages.
J Immunol; 116; 911-914.

Walker N S. (1977)
Mediation of macrophage cytolytic and phagocytic activities by antibodies of different classes and class specific Fc receptors.
J Immunol; 119; 367-373

Warr G A, (1980)
A macrophage receptor for mannose/glucosamine-glycoproteins of potential importance in phagocytic activity.
Biochem Biophys Res Comm; 93; 737-745.

Waytz P H, Douglas S D. (1979)
Increased antibody-dependent cell-mediated cytotoxicity by monocytes from patients with rheumatoid arthritis.
Arth & Rheum; 22; 490-494.

Weinstein A, Peters K, Brown D, Bluestone R (1972)
Metabolism of the third component of complement (C3) in patients with rheumatoid arthritis.
Arth & Rheum; 15; 49-56.

Weir D M, Blackwell C C, McLean A. (1981)
Impaired bacterial binding to peritoneal exudate cells from mice with alloxan induced diabetes.
J Clin Lab Immunol; 5; 37.

Weir D M, Graham L M, Ogmundsdottir H M. (1979)
Binding of mouse peritoneal macrophages to tumour cells by a lectin like macrophage receptor.
J Clin Lab Immunol; 2; 51-54.

Weisman M H, Zvaifler N J (1975)
Cryoimmunoglobulinaemia in rheumatoid vasculitis.
J Clin Invest; 56; 725-739.

Wellek B, Hahn H, Opfenkuch W. (1976)
Opsonising activities of IgG, IgM antibodies and C3b inactivator cleaved third component of complement in macrophage phagocytosis.
Agents & Actions; 6; 260-262.

Wenger M E and Bole G G. (1972a)
Nitroblue tetrazolium test in systemic lupus.
N Eng J Med; 287; 1150.

Wenger M E and Bole G G. (1972b)
Nitroblue tetrazolium dye reduction by peripheral leucocytes from patients with rheumatoid arthritis and systemic lupus erythematosus.
Arth & Rheum; 15; 459-60.

Wenger M E and Bole G G. (1973)
Nitroblue tetrazolium dye reduction by peripheral leucocytes from rheumatoid arthritis and systemic lupus erythematosus patients measured by a histochemical and spectrophotometric method.
J Lab Clin Med; 82; 513-21.

Werb Z, Banda M J, Jones P A. (1980a)
Degradation of connective tissue matrices by macrophages I. Proteolysis of elastin, glycoproteins and collagen by proteinases isolated from macrophages.
J Exp Med; 152; 1340-1357.

Werb Z, Bainton D F, Jones P A. (1980b)
Degradation of connective tissue matrices by macrophages III. Morphological and biochemical studies on extracellular, pericellular and intracellular events in matrix proteolysis by macrophages in culture.
J Exp Med; 152; 1537-1553.

Werb Z, Gordon S. (1975a)
Secretion of a specific collagenase by stimulated macrophages.
J Exp Med; 142; 346-360.

Werb Z, Gordon S. (1975b)
Elastase secretion by stimulated macrophages.
J Exp Med; 142; 361-377.

Werb Z, Mainardi C L, Vater C A, Harris E D. (1977)
Endogenous activation of latent collagenase by rheumatoid synovial cells.
N Eng J Med; 296; 1017-1023.

Whaley K (1980)
Biosynthesis of the complement components and the regulatory proteins of the alternative complement pathway by human peripheral blood monocytes.
J Exp Med; 151; 501-516.

Whaley K, Ruddy S. (1976)
Modulation of the alternative complement pathway by blH globulin.
J Exp Med; 144; 1147-1163.

Whaley K, El Ghobarey A. (1981)
"Complement" In: Carson-Dick, ed. Immunological aspects of rheumatology. Lancaster, MTP Press Chap 5; 113.

Whitelaw D M. (1972)
Observations on human monocyte kinetics after pulse labelling.
Cell Tissue Kin; 5; 311.

Whitelaw D M, Batho H F. (1972)
The distribution of monocytes in the rat.
Cell Tissue Kin; 5; 215.

Williams R C (Jr), Quie P G. (1971)
Opsonic activity of agammaglobulinemic human sera.
J Immunol; 106; 51-55.

Williams B D, Russell B A, Lockwood C M, Cotton C. (1979)
Defective reticuloendothelial function in rheumatoid arthritis.
Lancet; 1; 1311-14.

Wilson J G, Wong W W, Schur P H, Fearon D T. (1982)
Mode of inheritance of decreased C3b receptors on erythrocytes of patients with systemic lupus erythematosus.
N Eng J Med; 307; 981-6.

Winchester R J, Nunez-Roldan A. (1982)
Some genetic aspects of systemic lupus erythematosus.
Arth & Rheum; 25; 833-837.

Winkelhake H, Nicolson G. (1976)
Aglycosylantibody: effects of exoglucosidase treatments on autochthonous antibody survival time in the circulation.
J Biol Chem; 251; 1074-1080.

Woolley D E, Harris E D Jr, Mainardi C L, Brinckerhoff C E. (1978)
Collagenase immunolocalization in cultures of rheumatoid synovium.
Science 200; 773-5.

Woolley D E, Brinckerhoff C E, Mainardi C l, et al. (1979)
Collagenase production by rheumatoid synovial cells: morphological and immunohistochemical studies of the dendritic cell.
Ann Rheum Dis; 38; 262-70.

Wooley D E, Crossley M J, Evanson J M (1977)
Collagenase at sites of cartilage erosion in the rheumatoid joint.
Arth & Rheum; 20; 1231.

Wooley P H, Griffin J, Panayi G S, Batchelor J R, Weslsh K I, Gibson T J. (1980)
HLA-DR antigens and toxic reactions to sodium aurothiomalate and d-penicillamine in patients with rheumatoid arthritis.
N Eng J Med; 303; 300-302.

Yam L, Li C Y, Crosby W H. (1971)
Cytochemical identification of monocytes and granulocytes.
Am J Clin Path; 55; 283-90.

Yin H L, Stossel T P. (1980)
Purification and structural properties of gelsolin, a Ca++ activated regulatory protein of macrophages.
J Biol Chem; 255; 9490-9493.

Yonemasu K, Stroud R M (1971)
Rapid purification method for preparation of nonspecific antisera and for biomedical studies.
J Immunol; 106; 304.

Yoshiki T, Mellors R C, Strand M, August J T (1974)
The viral envelope glycoprotein of murine leukaemia virus and the pathogenesis of immune complex glomerulonephritis in New Zealand mice.
J Exp Med; 140; 1011-1023.

Young J D E, Unkeless J C, Kaback H R, Cohn Z A. (1983)
Mouse macrophage Fc receptors for IgG-gamma-2b/1 in artificial and plasma membrane vesicles functions as a ligand dependent ionophore.
Proc Nat Acad Sci USA; 80; 1636-1640.

Zubler R H, Nydegger U, Perrin L H, et al. (1976)
Circulating and intra-articular immune complexes in patients with rheumatoid arthritis. Correlation of (125)I-Clq binding activity with clinical and biological features of the disease.
J Clin Invest; 57; 1308.

Zuckerman S H, Douglas S H. (1979)
Dynamics of the macrophage plasma membrane.
Ann Rev Microbiol; 33; 267-307.

Zvaifler N J. (1981)
Aetiology and pathogenesis of systemic lupus erythematosus. In: Kelley W N, Harris E D, Ruddy S, Sledge C B, eds. Textbook of Rheumatology; ppl079-97; W B Saunders Company, Philadelphia.

Zvaifler N J (1973)
The immunopathology of joint inflammation in rheumatoid arthritis. Adv in Immunol; 16; 265-336.

Zvaifler N J (1982)
The pathogenesis of central nervous system manifestations of SLE Arth & Rheum; 25; 862-866.

Reprinted from the BRITISH MEDICAL JOURNAL,
27 June 1981, 282, 2081 - 2083

Evidence for defect of complement-mediated phagocytosis by monocytes from patients with rheumatoid arthritis and cutaneous vasculitis

N P HURST, G NUKI

Abstract

In-vitro measurements of the rate of monocyte phagocytosis of heat-killed yeast preopsonised in human AB serum from 14 patients with rheumatoid arthritis and 14 normal controls showed a significant reduction in five patients with active vasculitis but no change in nine with active arthritis alone. Further studies of complement- and Fc-mediated monocyte phagocytosis in which the rate constants (K_c and K_{Fc} respectively) were determined using complement-coated Saccharomyces cerevisiae and Candida albicans opsonised with IgG in monocytes from nine patients with rheumatoid vasculitis and 12 controls showed a significant reduction in K_c ($p < 0{\cdot}01$) but normal K_{Fc}. K_c was normal in three patients with inactive vasculitis. Low K_c was correlated with low serum C3 concentrations but not with Clq binding or anticomplementary activity, and no evidence of intracytoplasmic or membrane-bound immune complexes was detected in monocytes from patients with active vasculitis.

These results show that cutaneous vasculitis in rheumatoid arthritis is associated with selective impairment of

Rheumatic Diseases Unit, Department of Medicine, (WGH), University of Edinburgh, Northern General Hospital, Edinburgh EH5 2DQ

N P HURST, MB, MRCP, lecturer in rheumatology
G NUKI, MB, FRCP, professor of rheumatology

A7845/BMJ

complement-mediated monocyte phagocytosis, which does not appear to result from receptor blockade by immune complexes.

Introduction

Cutaneous vasculitis in rheumatoid arthritis is associated with hypocomplementaemia,[1] increased complement consumption,[2] large 19S IgM-containing circulating immune complexes,[3] and deposition of complement-containing immune complexes in small arterioles.[4] The episodic nature of clinical manifestations of vasculitis in rheumatoid arthritis suggests the possibility of intermittent saturation of immune clearance mechanisms. The cells thought to be responsible for clearance of circulating immune complexes are the mononuclear phagocytes of the spleen and liver.[5] As part of an investigation of mononuclear phagocyte function in rheumatoid arthritis we performed in-vitro measurements of monocyte phagocytosis in patients with uncomplicated rheumatoid arthritis, patients with rheumatoid cutaneous vasculitis, and normal controls.

Subjects and methods

Mononuclear cells were separated from 10 ml of venous blood using a density gradient,[6] washed, and resuspended in Hanks's balanced salt solution plus 0·1% gelatin. The concentration of monocytes present was determined using a rapid Coulter sizing technique[7] validated by comparison with differential cell counts performed with non-specific esterase stains.[8]

Heat-killed yeast were preopsonised in bulk, resuspended to 10^7/ml, and stored in aliquots in liquid nitrogen. Direct immunofluorescence verified that *Candida albicans* opsonised in fresh human serum was coated with complement (C3) and immunoglobulins (IgG, IgA, and IgM) while *Saccharomyces cerevisiae* in human serum was coated only with complement (C3). To provide yeast coated with IgG alone *C albicans* was opsonised in pooled human IgG.

Phagocytosis was measured by a modification of the method of Leijh *et al.*[9] Equal volumes (150 μl) of mononuclear cells and yeast particles were dispensed into six wells (400 μl), machined in a Teflon block, and incubated at 37°C under rotation. Aliquots of cell suspension were removed immediately after mixing and at timed intervals and diluted in counting fluid (2% acetic acid and gentian violet), and numbers of extracellular yeast remaining were counted in haemacytometers. The rate of phagocytosis was determined by the fall in yeast concentration. The method was validated using metabolic controls and electron microscopy. Pilot studies showed the kinetics of phagocytosis to be second order and that a rate constant K, which is a

measure of the efficiency of phagocytosis, could be obtained from the expression

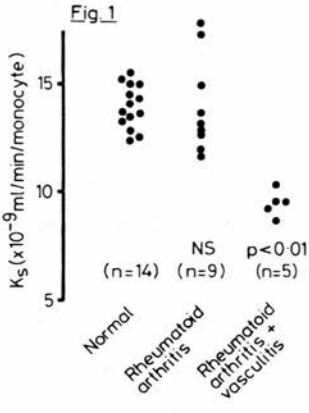
$$K = \frac{1}{t \times [M\varphi]} \times \ln \frac{No}{N_t} \text{ ml/min/monocyte}$$

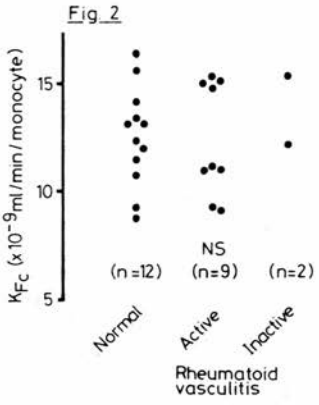
by measuring changes in the numbers of yeast (N) over a 20-minute interval (t) in the presence of a known monocyte concentration [Mφ]. The rate constant was measured initially using C *albicans* opsonised in human serum (K_s) from 14 normal controls and 14 patients with classical rheumatoid arthritis,[10] five of whom had active cutaneous vasculitis. Subsequently the rate constant was measured for Fc-mediated uptake (K_{Fc}) using *C albicans* opsonised in pooled human IgG and for C3-mediated uptake (K_c) using *S cerevisiae* opsonised in human serum from a group of 12 normal controls and nine patients with cutaneous vasculitis. Serum and EDTA plasma drawn at the time of the phagocytic assay were stored in liquid nitrogen for subsequent measurement of serum C3 and C4 concentrations (radial immunodiffusion; Seward Laboratories Immunostics), Clq binding activity,[11] and anticomplementary activity.[12]

Cytocentrifuge smears and washed cell suspensions of mononuclear cells were examined for the presence of intracytoplasmic immune complexes and membrane-associated immune complexes respectively by direct immunofluorescence using fluorescein conjugated antigammaglobulins and anti-β1C/β1A (Nordic Diagnostics).

Results

In the initial study using *C albicans* opsonised with both C3 and immunoglobulins there was no difference in the rate constant K_s between normal controls and patients with uncomplicated rheumatoid arthritis, but K_s was significantly reduced ($p < 0{\cdot}01$, rank sum test) in the patients with active vasculitis (fig 1). In subsequent studies, in which the rate constants for C3-mediated uptake (K_c) and Fc-mediated uptake (K_{Fc}) were measured separately, no change was found in K_{Fc} in the patients with vasculitis compared with the normal controls (fig 2) but K_c was significantly reduced ($p < 0{\cdot}01$, rank sum test) (fig 3). In three subjects whose vasculitis was judged to be clinically inactive K_c was in the normal range. All patients in whom K_c was low had low serum C3 concentrations, while the three subjects with inactive vasculitis and normal K_c had normal serum C3 concentrations (table). There was no clear correlation between a reduction in K_c and results of immune complex assays. All patients had high Clq binding activity both during and after vasculitic episodes and all had raised anticomplementary activity during vasculitis (table). Two of the three patients with inactive vasculitis had persistently raised anticomplementary activity.





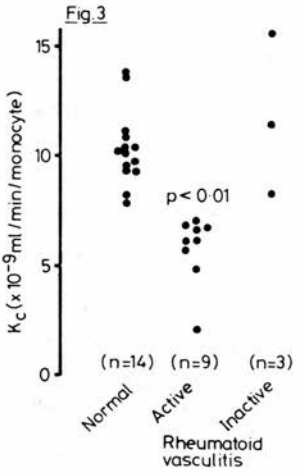


FIG 1—Monocyte phagocytic rate constant (K_s) in normal subjects and patients with rheumatoid arthritis and vasculitis.

FIG 2—Rate constant (K_{Fc}) for Fc-mediated phagocytosis in normal subjects and patients with active and inactive vasculitis.

FIG 3—Rate constant (K_c) for complement-mediated phagocytosis in normal subjects and patients with active and inactive vasculitis.

Complement-mediated phagocytosis (K_c), serum complement (C3 and C4) concentrations, and results of immune complex assays in patients with rheumatoid arthritis and rheumatoid cutaneous vasculitis

Patients	K_c (ml/min/monocyte)	C3 (mg/100 ml)	C4 (mg/100 ml)	Clq binding activity (%)	Anticomplementary activity titre
Uncomplicated rheumatoid arthritis	Normal	>123	>33	0-90	0
Active vasculitis (n = 9)	Low	64-115	10-26	72-99	2-16
Inactive vasculitis (n = 3)	Normal	>123	21-37	58-99	0-2

No intracytoplasmic or membrane-associated immune complexes were found in monocytes from four patients with active vasculitis and low K_c.

Discussion

These studies appear to show for the first time selective functional depression of complement-mediated monocyte phagocytosis in patients with rheumatoid vasculitis. The synchronous depression of K_c and serum C3 concentration in the presence of high Clq binding activity and anticomplementary activity might suggest that saturation of complement-mediated clearance mechanisms is associated with the appearance of clinical vasculitis in rheumatoid arthritis. A similar defect limited to complement-mediated clearance of coated red cells has recently been shown in vivo in patients with primary biliary cirrhosis,[13] a disease that is associated with circulating complexes containing IgM and IgG and with increased complement catabolism.[14] Large complexes containing 19S complement-fixing IgM are also found in rheumatoid vasculitis, but the relation is not constant and the appearance of vasculitis unpredictable.[3] The finding that complement-receptor-mediated phagocytosis is depressed in these patients while Fc-receptor function is unchanged might suggest that these complexes are binding preferentially to complement receptors and that binding of IgG Fc ligands is sterically inhibited by the presence of IgM rheumatoid factor. However, attempts to show the presence of surface-attached or intracytoplasmic immune complexes in monocytes from these patients by direct immunofluorescence have been unsuccessful.

An alternative explanation for these findings may be that rheumatoid vasculitis is associated with a change in the composition of the peripheral monocyte population and that the defect in complement-mediated phagocytosis is unrelated to the binding of immune complexes to these cells. Previous work[15] has shown the presence of circulating functionally immature monocyte precursors in patients with rheumatoid arthritis and systemic lupus erythematosus. We are currently exploring the relative maturation of complement and Fc-receptor function in monocytes from patients with these diseases.

Whatever the basis for the underlying defect of complement-mediated phagocytosis it could have direct relevance to the pathogenesis of rheumatoid vasculitis.

Assays of Clq binding activity and anticomplementary activity were kindly performed by Dr T B Wallington, of the South-west Regional Transfusion Centre, Bristol, and complement assays by

Dr K C Watson, of the department of bacteriology, Western General Hospital, Edinburgh. The work was supported by grants from the Arthritis and Rheumatism Council.

Requests for reprints should be addressed to Dr N P Hurst, Rheumatic Diseases Unit, Northern General Hospital, Ferry Road, Edinburgh EH5 2DQ.

References

1 Morgan ES, Cass RM, Jacox RF, Vaughan JH. A study of the relations of seronegative and seropositive rheumatoid arthritis to each other and to necrotising vasculitis. *Am J Med* 1969;**47**:23-35.

2 Weinstein A, Peters K, Brown D, Bluestone R. Metabolism of the third component of complement (C3) in patients with rheumatoid arthritis. *Arthritis Rheum* 1972;**15**:49-56.

3 Weisman MH, Zvaifler NJ. Cryoimmunoglobulinaemia in rheumatoid vasculitis. *J Clin Invest* 1975;**56**:725-39.

4 Conn DL, McDuffie FC, Dyck PJ. Immunopathologic study of sural nerves in rheumatoid arthritis. *Arthritis Rheum* 1972;**15**:135-43.

5 Benacerraf B, Sebestyen M, Cooper NS. The clearance of antigen-antibody complexes from the blood by the reticulo endothelial system. *J Immunol* 1959;**82**:131-7.

6 Böyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 1968;**21**, suppl 97:77-89.

7 Loos H, Blok-Schut B, Kipp B, Van Doorn R, Meerhof L. Size, distribution, electronic recognition and counting of human blood monocytes. *Blood* 1976;**48**:743-53.

8 Yam LT, Li CY, Crosby WH. Cytochemical identification of monocytes and granulocytes. *Am J Clin Pathol* 1971;**55**:283-90.

9 Leijh PCT, Van den Barselaar MT, Van Furth R. Kinetics of phagocytosis and intracellular killing of Candida albicans by human granulocytes and monocytes. *Infect Immun* 1977;**17**:313-8.

10 Ropes MW, Bennett GA, Cobb S, Jacox R, Jessar RA. Diagnostic criteria for rheumatoid arthritis. 1958 revision. *Ann Rheum Dis* 1959;**18**:49-53.

11 Zubler RH, Nydegger U, Perrin LH, *et al.* Circulating and intra-articular immune complexes in patients with rheumatoid arthritis. Correlation of ^{125}I-C1q binding activity with clinical and biological features of the disease. *J Clin Invest* 1976;**57**:1308.

12 Verrier-Jones J, Cumming RH. Tests for circulating immune complexes. In: Thomson RA. *Techniques in clinical immunology*. Oxford: Blackwell, 19:136-57.

13 Jaffe CJ, Vierling JM, Jones EA, Lawley TJ, Frank MF. Receptor specific clearance by the reticulo endothelial system in chronic liver diseases. Demonstration of defective C3b-specific clearance in primary biliary cirrhosis. *J Clin Invest* 1978;**62**:1069-77.

14 Wands JR, Dienstag JL, Bhan AK, Feller ER, Isselbacher KJ. Circulating immune complexes and complement activation in primary biliary cirrhosis. *N Engl J Med* 1978;**298**:233-7.

15 Horwitz DA, Steagall RV Jr. The development of macrophages from large mononuclear cells in the blood of patients with inflammatory disease. *J Clin Invest* 1972;**51**:760-8.

(*Accepted 14 May 1981*)

Printed in Great Britain by Bourne Offset (Iver) Ltd., Iver, Bucks.